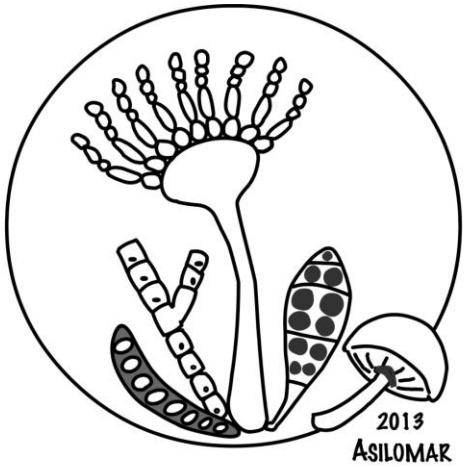
Role of MAP kinase pathways in the pathogenicity of the wheat pathogen Mycosphaerella graminicola.

Elisabetta E. Marchegiani, Julie Vallet, Sian Deller, Marc-Henri M.-H. Lebrun



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March 12 – 17, 2013

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27th Fungal Genetics Conference

March 12 – 17, 2013
Asilomar Conference Grounds
Program and Abstracts Volume

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Please note: The program book from the 27th Fungal Genetics Conference is published as a supplement to the Fungal Genetics Reports. Abstracts will be available on the FGSC website and may be cited as follows: Fungal Genetics Reports 60(Suppl): Abstract #

SCHEDULE OF EVENTS

Tuesday, March 12

2:00 pm - 10:00 pm	Registration	Chapel
6:00 pm - 7:00 pm	Dinner	Crocker Hall
7:30 pm - 10:30 pm	Opening Mixer	Merrill Hall

Wednesday, March 13

7:30 am - 8:30 am	Breakfast	Crocker Hall
8:00 am – 5:00 pm	Registration	Chapel

8:30 am - 12:00 noon **Plenary Session I** **Merrill Hall and Chapel**

Metabolic Pathways: Cell Growth, Pathogenesis and Bioenergy *Chair:* Louise Glass

Robert A. Cramer	**Hypoxia and Mechanisms of Human Fungal Pathogenesis: To Air or Not to Air?**
Audrey P. Gasch	**Tackling biofuel bottlenecks through genome wide association studies in *Saccharomyces cerevisiae***
Kim E. Hammond-Kosack	**Exploring the metabolome of cereal infecting fusaria**
Bernard Henrissat	**Carbohydrate-active enzymes in fungal genomes**
Adrian Tsang	**Genome-wide approaches to identify and characterize lignocellulolytic enzymes**

12:00 noon - 1:00 pm Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

Ad hoc Workshops – Box lunches will be available for those attending the session.

12:15 pm - 1:30 pm	Fungal Genome Tools	Chapel
1:30 pm - 1:45 pm	One Name = One Fungus	Chapel

3:00 pm - 6:00 pm **Concurrent Sessions I**

Cell Signaling Involved in Fungal Development and Pathogenesis	Naweed Naqvi and Stefanie Pöggeler	**Merrill Hall**
Genetics and Genomics of Interactions with Bacteria, Insects and Plants	Nemat Keyhani and Christian Hertweck	**Chapel**
Membrane Trafficking and Molecular Organization	Vicky Sophianopoulou and Gero Steinberg	**Heather Fred Farr**
Genome Defense, Epigenetics and RNAi	Patrick Shiu and Sven Saupe	**Forum**
Genomics and Mycorrhizae	Anders Tunlid and Tom Bruns	**Kiln**
Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism	Richard Wilson and Ronald de Vries	**Nautilus**
Education, Outreach, and Professional Development	Steven Denison and Mimi Zolan	**Scripps**

6:00 pm - 7:00 pm Dinner Crocker Hall

7:30 pm - 10:30 pm **Poster Session I and Exhibits** Fireside Pavilion
ODD numbered posters from 7:30 – 8:30 and EVEN numbered posters 8:30 – 9:30.

Poster Number	Topic
1-68	Biochemistry and Metabolism
69-220	Cell Biology and Development
351-353	Education and Professional Development

Thursday, March 14

7:30 am - 8:30 am	Breakfast	Crocker Hall
8:30 am – 5:00 pm	Registration	Surf and Sand

8:30 am - 12:00 noon **Plenary Session II** **Merrill Hall and Chapel**

Organismic Molecular Interactions *Chair:* Nick Talbot

Yong-Hwan Lee	**Large-scale Biology for Fungal Pathogenicity in *Magnaporthe oryzae***
Antonio Di Pietro	**MAPK-mediated control of infectious growth in *Fusarium oxysporum***
Peter Dodds	**Analysis of effector proteins from flax rust and wheat stem rust**
Francine Govers	**Dissecting *Phytophthora* blight; making sense out of signalling, effectors and host targets**
Alexandra C. Brand	**Understanding directional growth in fungi**

12:00 noon - 1:00 pm Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

12:15 pm - 1:15 pm	GSA Careers Luncheon	Crocker Hall
Ad hoc Workshops	Box lunches will be available for those attending the sessions.	
12:15 pm - 1:30 pm	Neurospora Business Meeting	Chapel
12:30 pm - 2:00 pm	JGI Sequencing and Analysis Tools and Initiatives	Merrill Hall

3:00 pm - 6:00 pm **Concurrent Sessions II**

Cool Tools for Fungal Biology	Miguel Penalva and Kevin McCluskey	**Merrill Hall**
Fungi and Evolutionary Theory	Hanna Johannesson and Duur Aanen	**Chapel**
Cytoskeleton, Motors, and Intracellular Transport	Samara Reck-Peterson and Ping Wang	**Heather**
Nucleic Acid-Protein Interactions that Impact Transcription and Translation	Michael Freitag and Mark Caddick	**Fred Farr Forum**
Interactions between Fungi and Animals	Neil Gow and Clarissa Nobile	**Kiln**
Fungal Volatiles and Organic Compounds as Signaling Agents	Joan Bennett and Richard Splivallo	**Nautilus**
Genomics and Biochemistry of Degradation of Complex Molecules in the Environment	Jonathan Walton and Dan Cullen	**Scripps**

6:00 pm - 7:00 pm	Dinner	Crocker Hall
7:30 pm - 10:30 pm	**Poster Session II and Exhibits**	Fireside
ODD numbered posters from 7:30 – 8:30 and EVEN numbered posters from 8:30 – 9:30		Pavilion

Poster Number	Topic
221-350	Comparative and Functional Genomics
354-475	Gene Regulation

SCHEDULE OF EVENTS

Friday, March 15

7:30 am - 8:30 am	Breakfast	Crocker Hall
8:30 am – 5:00 pm	Registration	Surf and Sand

8:30 am - 12:00 noon **Plenary Session III** **Merrill Hall and Chapel**

Sensing, Cell Biology and Development *Chair:* Michelle Momany

Meritxell Riquelme	**The illuminated Spitzenkörper of *Neurospora crassa*: tracking and tracing secretory vesicles**
Joseph Heitman	**Evolution of sexual reproduction: A view from the Fungal Kingdom**
Michael Brunner	**Metabolic compensation of the Neurospora clock by a glucose-dependent feedback of the circadian repressor CSP1 on the core oscillator**
Stephen Osmani	**Integration of the fungal cell cycle with growth and development**
Gregory Jedd	**A Neurospora cell-free system reconstitutes peroxisome membrane protein synthesis and organelle-specific targeting**

12:00 noon - 1:00 pm Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

Ad hoc Workshops Box lunches will be available for those attending the sessions.

12:15 pm - 1:30 pm	FungiDB	Kiln
12:15 pm - 1:30 pm	Magnaporthe Comparative Genomics	Chapel

3:00 pm - 6:00 pm **Concurrent Sessions III**

Pathogenic Signaling via Effector Proteins	Brett Tyler and Sebastien Duplessis	**Merrill Hall**
Cell Wall, Polarity and Hyphal Tip Growth	Stephan Seiler and Ernestina Castro-Longoria	**Chapel**
Sexual Regulation and Evolution in the Fungi	Frances Trail and Nicolas Corradi	**Heather**
Oxidative Stress, ROS Signaling and Adaptation to Hypoxia	Geraldine Butler and Barry Scott	**Fred Farr Forum**
Phylogenomics	Jason Stajich and Joey Spatafora	**Kiln**
Synthetic Biology	Nancy Keller and Peter Punt	**Nautilus**
Fungicides and Antifungal Compounds	Daniele Debieu and Paul Verweij	**Scripps**

6:00 pm - 7:00 pm	Dinner	Crocker Hall
7:00 pm - 8:00 pm	GSA Education Special Interest Group Mixer	Surf and Sand

7:30 pm - 10:30 pm **Poster Session III and Exhibits** Fireside
ODD numbered posters 7:30 – 8:30 and EVEN numbered posters 8:30 – 9:30. Pavilion

Poster Number	Topic
476 – 639	Pathogenic and Mutualistic Interactions
640 - 688	Population and Evolutionary Genetics
714 - 741	Other Topics

Saturday, March 16

7:30 am - 8:30 am	Breakfast	Crocker Hall
9:00 am – 12:00 noon	Registration	Surf and Sand

8:30 am - 12:00 noon **Plenary Session IV** **Merrill Hall and Chapel**

Functional Ecology and Fungal Communities *Chair:* Jim Anderson

Tatiana Giraud	**Mechanisms allowing the formation of new fungal pathogenic species on novel hosts, causing emerging diseases**
B. D. Lindahl	**The decisive role of mycorrhizal fungi as regulators of carbon sequestration in boreal forest ecosystems**
Edward J. Louis	**Population Genomics of Saccharomyces Yeasts: Ecology and Adaptation**
Marc-André Selosse	**The mycorrhizal symbiosis as a network linking plants**
Eva H. Stukenbrock	**Unraveling speciation and specialization processes in plant pathogenic fungi using comparative population genomics**

12:00 noon - 1:00 pm Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

2:00 pm - 5:00 pm **Concurrent Session IV**

Parallels between Fungal Pathogens of Plants and Animals	Barbara Howlett and Axel Brakhage	**Merrill Hall**
Secondary Metabolism	Gillian Turgeon and Bettina Tudzynski	**Chapel**
Light Sensing and Circadian Rhythms	Luis Larrondo and Reinhard Fischer	**Heather**
Fungal Evo-Devo	Steve Harris and Brian Shaw	**Fred Farr Forum**
Environmental Metagenomics	Chris Schadt and Betsy Arnold	**Kiln**
Dimorphic Transitions	Anne Dranginis and Alex Andrianopoulos	**Nautilus Scripps**
Tropic Growth and Fusion	Andre Fleissner and Nick Read	

5:30 pm - 5:45 pm	Poster Awards	Merrill Hall and Chapel
5:45 pm - 6:30 pm	**Perkins/Metzenberg Lecture**: Regine Kahmann, Max Planck Institute for Terrestrial Microbiology	Merrill Hall and Chapel
6:30 pm - 8:30 pm	Closing Banquet	Crocker Hall
8:30 pm - 12:00 am	Closing Party featuring The Amplified DNA Band	Merrill Hall
8:30 pm - 12:00 am	Quiet Alternative	Surf and Sand

Sunday, March 17

7:30 am - 8:30 am	Breakfast	Crocker Hall

GENERAL INFORMATION

Registration and Information Desk
The Conference registration desk will be open according to the following schedule:

Date	Time	Location
Tuesday, March 12	2:00 pm – 10:00 pm	Surf and Sand
Wednesday, March 13	8:00 am – 5:00 pm	Surf and Sand
Thursday, March 14	8:30 am – 5:00 pm	Surf and Sand
Friday, March 15	8:30 am – 5:00 pm	Surf and Sand
Saturday, March 16	9:00 am – 12:00 noon	Surf and Sand

Instructions for Speakers
Please arrive 45 minutes before the beginning of your session with your USB flash drive to load your presentation on the laptop computer. Label your presentation with your last name and presentation number, i.e. Chen12. You do not need to bring your laptop to the meeting room. However there will be connections for presenters to use their own laptops. Speakers using their own laptops must have a VGA HD 15pin female output.

Poster Sessions
All posters will be displayed in the garage under the Fred Farr Forum. Please set up your poster immediately after lunch the day of your poster session. Two authors will share a 4 x 8 poster board. All abstracts will be up for one day. Authors of ODD numbered posters should be at their poster from 7:30 pm – 8:30 pm and authors of even numbered posters should be at the posters from 8:30 pm – 9:30 pm. Authors will present according to the following schedule:

Topic	# Range	Wednesday, Posters I	Thursday, Posters II	Friday, Posters III
Biochemistry and Metabolism	**1 - 68**	1 – 68		
Cell Biology and Development	**69 - 220**	69 - 220		
Comparative and Functional Genomics	**221 - 350**		221 - 350	
Education and Professional Development	**351 - 353**	351 - 353		
Gene Regulation	**354 - 475**		354 - 475	
Pathogenic and Mutualistic Interactions	**476 - 639**			476 – 639
Population and Evolutionary Genetics	**640 - 688**			640 - 688
Other Topics	**689 - 741**	689 - 713		714 - 741

All presenters should remove their abstracts at the end of their poster session. After that time, remaining posters will be removed and may be lost or thrown away. The meeting does not take responsibility for posters that are not removed on time.

Internet Access
Complimentary internet access is available in the Fireside Pavilion. Instructions to connect to wifi:

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Wednesday, March 13 3:00 PM–6:00 PM
Merrill Hall

Cell Signaling Involved in Fungal Development and Pathogenesis

Co-chairs: Naweed Naqvi and Stefanie Pöggeler

Abstracts for this session begin on page 29.

3:00 - 3:20
Alexander V. Michkov
Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor.

3:20 - 3:40
Jae-Hyuk Yu
The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in *Aspergillus*.

3:40 - 4:00
Oezguer Bayram
The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism.

4:00 - 4:20
Ines Teichert
The developmental PRO40/SOFT protein participates in signaling via the MIK1/MEK1/MAK1 module in *Sordaria macrospora*.

4:20 - 4:40 **Break**

4:40 - 5:00
Linqi Wang
A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrinal Signaling.

5:00 - 5:20
JinRong Xu
Surface recognition and appressorium morphogenesis in Magnaporthe oryzae.

5:20 - 5:40
Marie Nishimura
Plant cues promote stealth infection in fungal plant pathogens.

5:40 - 6:00
Andrea Herrmann
Unravelling the GTPase polarity complex in *Claviceps purpurea*.

Wednesday, March 13 3:00 PM–6:00 PM
Chapel

Genetics and Genomics of Interactions with Bacteria, Insects and Plants

Co-chairs: Nemat Keyhani and Christian Hertweck

Abstracts for this session begin on page 32.

3:00 - 3:20
M. J. Bidochka
Endophytic insect parasitic fungi feed insect-derived nitrogen to plants.

3:20 - 3:40
Rusty J. Rodriguez
Genotype-Environment Interactions and the Interplay Between Climate Change and Plant-Fungal Symbioses.

3:40 - 4:00
Kirstin Scherlach
Chemical mediators of pathogenic and mutualistic bacterial-fungal interactions.

4:00 - 4:20
Chengshu Wang
Comparative genomic analysis of entomopathogenic fungi.

4:20 - 4:40 **Break**

4:40 - 5:00
Morten Schiøtt
Synergistic interactions between leaf-cutting ants and their fungal symbiont facilitate degradation of plant substrate.

5:00 - 5:20
Charissa de Bekker
Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains.

5:20 - 5:40
Artemio Mendoza
Trichoderma rhizosphere's competency, endophytism and plant communication: A molecular approach.

5:40 - 6:00
Markus Künzler
Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance.

Wednesday, March 13 3:00 PM–6:00 PM
Heather

Membrane Trafficking and Molecular Organization

Co-chairs: Vicky Sophianopoulou and Gero Steinberg

Abstracts for this session begin on page 35.

3:00 - 3:20
Barbara Valent
Distinct secretion systems operate during biotrophic invasion by the rice blast fungus, *Magnaporthe oryzae*.

3:20 - 3:40
Yujiro Higuchi
The cellular role of early endosome motility in *Ustilago maydis*.

3:40 - 4:00
George Diallinas
The arrestin-like protein ArtA is essential for ubiquitylation and endocytosis of the UapA transporter in response to both broad-range and specific signals.

4:00 - 4:20
Guido Grossmann
Escaping the hustle - zones of differential protein turnover in the yeast plasma membrane.

4:20 - 4:40 **Break**

5:00 - 5:20
Samara Reck-Peterson
Whole-genome sequencing identifies novel alleles of genes required for organelle distribution and motility in *Aspergillus nidulans*.

5:20 - 5:40
Rosa R. Mouriño-Pérez
Dynamics of exocytic markers and cell wall alterations in an endocytosis mutant of *Neurospora crassa*.

5:40 - 6:00
Barry J. Bowman
"The vacuole" of Neurospora crassa may be composed of multiple compartments with different structures and functions.

Wednesday, March 13 3:00 PM–6:00 PM
Fred Farr Forum

Genome Defense, Epigenetics and RNAi

Co-chairs: Patrick Shiu and Sven Saupe

Abstracts for this session begin on page 37.

3:00 - 3:20
Patrick K. T. Shiu
Meiotic silencing by unpaired DNA in *Neurospora*.

3:20 - 3:40
Zhenyu Zhang
Mechanism of quelling, a small RNA-mediated gene silencing pathway.

3:40 - 4:00
Xuying Wang
SIS, a sex genome defense mechanism operating in *Cryptococcus neoformans*.

4:00 - 4:20
Asen Daskalov
Fungi use prion folds for signal transduction processes involving STAND proteins.

4:20 - 4:40 **Break**

4:40 - 5:00
Haoping Liu
Regulation of white and opaque cell-type formation in Candida albicans by H3K56 acetylation and nucleosome assembly factors CAF-1 and HIR.

5:00 - 5:20
Matthew Z. Anderson
Epigenetic Regulation of Subtelomeric Gene Noise in *Candida albicans*.

5:20 - 5:40
Zachary A. Lewis
Chromatin regulation of genome stability.

5:40 - 6:00
Shinji Honda
Opposing activities of the HCHC and DMM complexes maintain proper DNA methylation in *Neurospora crassa*.

Wednesday, March 13 3:00 PM–6:00 PM
Kiln

Genomics and Mycorrhizae

Co-chairs:

Abstracts for this session begin on page 39.

3:00 - 3:20
A. Kohler
The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq.

3:20 - 3:40
Jaqueline Hess
Transposable element dynamics in the *Amanita*: insights on the evolution of genome architecture accompanying the transition from saprotrophic to ectomycorrhizal ecologies.

3:40 - 4:00
Alga Zuccaro
Broad compatibility in the root endophyte *Piriformospora indica* is associated with host-adapted colonization strategies.

4:00 - 4:20
Anders P. V. Tunlid
Examining the saprotrophic ability of ectomycorrhizal fungi using genomics, transcriptomics and spectroscopy.

4:20 - 4:40 **Break**

4:40 - 5:00
Nils OS Högberg
Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots.

5:00 - 5:20
Stephen J. Mondo
Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis.

5:20 - 5:40
Alija Mujic
A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon.

5:40 - 6:00
H.-L. Liao
Metatranscriptomic analysis of ectomycorrhizal root clusters in *Pinus taeda*: new methodologies for assessing functional gene expression *in situ*.

Wednesday, March 13 3:00 PM–6:00 PM
Nautilus

Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism

Co-chairs: Richard Wilson and Ronald de Vries

Abstracts for this session begin on page 42.

3:00 - 3:20
Carl R. Fellbaum
The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhiza.

3:20 - 3:40
Jessie Fernandez
Mechanisms of adaptation to host rice cells by the blast fungus.

3:40 - 4:00
Sylvia Klaubauf
Similar is not the same: Differences in the function of the (hemi-) cellulolytic regulator XlnR (Xlr1/Xyr1) in filamentous fungi.

4:00 - 4:20
Richard B. Todd
Regulating the *Aspergillus nidulans* global nitrogen transcription factor AreA.

4:20 - 4:40 **Break**

4:40 - 5:00
Miia R. Mäkelä
Transcriptional analysis of oxalate degradation in the white rot basidiomycete *Dichomitus squalens*.

5:00 - 5:20
Gesabel Y. Navarro Velasco
TOR-mediated control of virulence functions in the trans-kingdom pathogen *Fusarium oxysporum*.

5:20 - 5:40
Firoz Shah
Transcriptional regulation of peptidases and nitrogen transporters during the assimilation of organic nitrogen by the ectomycorrhizal fungi *Paxillus involutus*.

5:40 - 6:00
Michael Hynes
Regulation of glycolysis and gluconeogenesis by antisense transcription in *Aspergillus nidulans*?

Wednesday, March 13 3:00 PM–6:00 PM
Scripps

Education, Outreach, and Professional Development

Co-chairs: Steven Denison and Mimi Zolan

Abstracts for this session begin on page 45.

3:00 - 3:20
Michael Koonce
Centrosome-Nuclear Disconnect Creates Mitotic Chaos in a Closed Mitosis System.

3:20 - 3:40
Claire Burns
Using Fungal Barcoding to Introduce Non-science Majors to Scientific Research.

3:40 - 4:00
Andrea Gargas
ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis.

4:00 - 4:20
Patricia J. Pukkila
Wearing two hats: Tips for combining commitments to research and to university-wide initiatives in education.

4:20 - 4:40 **Break**

4:40 - 5:00
Virginia K. Hench
Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread.

5:00 - 5:20
Relly Brandman
MOOCs: Education for Everyone.

5:20 - 5:40
Break into groups to discuss promises and pitfalls of online courses.

5:40 - 6:00
Panelists Relly Brandman and Heaher Hallen-Adams respond to questions and comments from the working groups.

Thursday, March 14 3:00 PM–6:00 PM
Merrill Hall

Cool Tools for Fungal Biology

Co-chairs: Miguel Penalva and Kevin McCluskey

Abstracts for this session begin on page 47.

3:00 - 3:20
S. E. Baker
The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology.

3:20 - 3:40
Aric E. Wiest
Development and utilization of arrayed mutant sets for yeasts and filamentous fungi.

3:40 - 4:00
Minou Nowrousian
Sequencing-based solutions to identify and characterize fungal developmental genes.

4:00 - 4:20
Susan Kaminskyj
***Aspergillus nidulans* as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug resistance mutations.**

4:20 - 4:40 **Break**

4:40 - 5:00
David L. Joly
Illumina-based genetic linkage map for wheat leaf rust.

5:00 - 5:20
Miguel Penalva
Peering into the secret-ory life of *Aspergillus nidulans* with a little help from classical genetics.

5:20 - 5:40
Patricia J. Pukkila
Domains of meiotic DNA recombination and gene conversion in *Coprinopsis cinerea* (*Coprinus cinereus*).

5:40 - 6:00
Xin Xiang
A Hook protein is critical for dynein-mediated early endosome movement in Aspergillus nidulans.

Thursday, March 14 3:00 PM–6:00 PM
Chapel

Fungi and Evolutionary Theory

Co-chairs: Hanna Johannesson and Duur Aanen

Abstracts for this session begin on page 49.

3:00 - 3:20
Anne Pringle
Reaching the wind: the fluid mechanics of spore discharge, and potential for dispersal mechanisms to shape the evolution of sporocarp and spore morphologies.

3:20 - 3:40
Jennifer L. Anderson
***Neurospora tetrasperma* mating-type chromosomes: Testing hypotheses on the effects of degeneration and introgression on performance.**

3:40 - 4:00
Bart Nieuwenhuis
Nuclear arms races: sexual selection for masculine mushrooms.

4:00 - 4:20
James B. Anderson
Genome-wide mutation dynamic within a long-lived individual of Armillaria.

4:20 - 4:40 **Break**

4:40 - 5:00
C. Angelard
Rapid genetic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation.

5:00 - 5:20
Pierre Grognet
Meiotic Drive: A Single Gene Conferring Killing and Resistance in Fungal Spore Killer.

5:20 - 5:40
Pierre Gladieux
Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta.

5:40 - 6:00
Georgiana May
Ecological context in symbioses: when is your enemy also your friend?

Thursday, March 14 3:00 PM–6:00 PM
Heather

Cytoskeleton, Motors, and Intracellular Transport

Co-chairs: Samara Reck-Peterson and Ping Wang

Abstracts for this session begin on page 51.

3:00 - 3:20
Gero Steinberg
The molecular basis of extended dynein run-length.

3:20 - 3:40
Martin Egan
The role of microtubule-based motors in the spatiotemporal control of autophagy.

3:40 - 4:00
Sebastian Baumann
Microtubule-dependent co-transport of mRNPs and endosomes.

4:00 - 4:20
Flora Banuett
Role of *tea1* and *tea4* homologs in cell morphogenesis in *Ustilago maydis*.

4:20 - 4:40 **Break**

4:40 - 5:00
Yainitza Hernandez-Rodriguez
***Aspergillus nidulans* septin interactions and post-translational modifications.**

5:00 - 5:20
Connie B. Nichols
Altered Ras1 trafficking impairs the pathogencity of *Cryptococcus neoformans*.

5:20 - 5:40
Karen Stephenson
Quantification of the thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography.

5:40 - 6:00
P. Philippsen
Dynein drives oscillatory nuclear movements in the phytopathogenic fungus *Ashbya gossypii* and prevents nuclear clustering.

Thursday, March 14 3:00 PM–6:00 PM
Fred Farr Forum

Nucleic Acid-Protein Interactions that Impact Transcription and Translation

Co-chairs: Michael Freitag and Mark Caddick

Abstracts for this session begin on page 53.

3:00 - 3:20
Koon Ho Wong
ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi.

3:20 - 3:40
Jay C. Dunlap
Regulatory Networks Governing Global Responses to Changes in Light and Time.

3:40 - 4:00
L. F. Larrondo
Protein Binding Microarrays and *high-throughput* real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in *Neurospora crassa*.

4:00 - 4:20
Ane Sesma
Ending messages: alternative polyadenylation in filamentous fungi.

4:20 - 4:40 **Break**

4:40 - 5:00
Amanda L. Misener Bloom
Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in *Cryptococcus neoformans*.

5:00 - 5:20
Johannes Freitag
Dual targeting of glycolytic enzymes by alternative splicing and translational read-through.

5:20 - 5:40
Mian Zhou
Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY.

5:40 - 6:00
Michael Feldbrugge
A transcriptome-wide view on microtubule-dependent mRNA transport.

Thursday, March 14 3:00 PM–6:00 PM
Kiln

Interactions between Fungi and Animals

Co-chairs: Neil Gow and Clarissa Nobile

Abstracts for this session begin on page 55.

3:00 - 3:20
Elaine M. Bignell
Elicitation of host damage occurs in a temporally programmed manner during Aspergillus fumigatus infections.

3:20 - 3:40
Stuart Levitz
Exploiting innate recognition of fungi for vaccine development.

3:40 - 4:00
Jose C. Perez
Regulatory circuits governing Candida albicans proliferation in a mammalian host.

4:00 - 4:20
Judith Berman
Dramatic ploidy change as an adaptive strategy in Candida albicans...

4:20 - 4:40 **Break**

4:40 - 5:00
Yen-Ping Hsueh
Nematode-trapping fungi eavesdrop on nematode pheromones.

5:00 - 5:20
Xiaorong Lin
A morphogenesis regulator controls cryptococcal neurotropism.

5:20 - 5:40
M. Brock
Sit and wait: Special features of *Aspergillus terreus* in macrophage interactions and virulence.

5:40 - 6:00
Dawn Thompson
The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans.

Thursday, March 14 3:00 PM–6:00 PM
Nautilus

Fungal Volatiles and Organic Compounds as Signaling Agents

Co-chairs: Joan Bennett and Richard Splivallo

Abstracts for this session begin on page 58.

3:00 - 3:20
Birgit Piechulla
Fungi reacting to rhizobacterial volatiles.

3:20 - 3:40
Seogchan Kang
Enhancement of plant growth and stress resistance by Fusarium volatile organic compounds: A novel mechanism mediating plant-fungal interactions.

3:40 - 4:00
Jessica C. Hargarten
The Role of Quorum-sensing Molecules in Interactions between *Candida albicans* and its Host.

4:00 - 4:20
Vong shian Simon Ip Cho
Innate Immunity in *Fusarium graminearum*.

4:20 - 4:40 **Break**

4:40 - 5:00
Lea Atanasova
The *Trichoderma reesei* polyketide synthase gene *pks1* is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness.

5:00 - 5:20
Richard Hung
Semiochemicals and signaling: plant responses to *Trichoderma* volatile organic compounds.

5:20 - 5:40
El Ghalid Mennat
Identification of chemoattractant compounds from tomato root exudate that trigger chemotropism in *Fusarium oxysporum*.

5:40 - 6:00
Richard Splivallo
The mixed fungal and bacterial origin of truffle aroma.

Thursday, March 14 3:00 PM–6:00 PM
Scripps

Genomics and Biochemistry of Degradation of Complex Molecules in the Environment

Co-chairs: Jonathan Walton and Dan Cullen

Abstracts for this session begin on page 61.

3:00 - 3:20
K. Igarashi
Fungal transcriptome as database for proteome and refinement tool of gene annotation.

3:20 - 3:40
Irina S. Druzhinina
Developmental regulation and cellulase gene expression in *Trichoderma reesei*.

3:40 - 4:00
D. Floudas
Parallel losses of genes associated with saprotrophy in ectomycorrhizal Agaricomycotina lineages.

4:00 - 4:20
Emma Master
Co-expression analysis of *Phanerochaete carnosa* during growth on hardwood and softwood species to predict proteins with unknown function relevant to biomass conversion.

4:20 - 4:40 **Break**

4:40 - 5:00
Yitzhak Hadar
Functional Analysis of the *Pleurotus ostreatus* Manganese-Peroxidase Gene Family.

5:00 - 5:20
Monika Schmoll
Carbon source and light dependent regulation of gene clusters in *Trichoderma reesei* (*Hypocrea jecorina*).

5:20 - 5:40
Chiaki Hori
Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay.

5:40 - 6:00
Alex Lichius
Transcription factor shuttling during cellulase induction in *Trichoderma reesei*.

Friday, March 15 3:00 PM–6:00 PM
Merrill Hall

Pathogenic Signaling via Effector Proteins

Co-chairs: Brett Tyler and Sebastien Duplessis

Abstracts for this session begin on page 64.

3:00 - 3:20
Marie-Cecile Caillaud
Dissecting nuclear immunity using Arabidopsis downy mildew effector as probes.

3:20 - 3:40
Claire Veneault-Fourrey
The mutualistic fungus *Laccaria bicolor* uses the effector protein MiSSP7 to alter host jasmonate signaling and establish symbiosis.

3:40 - 4:00
Shiv D. Kale
Identification and characterization of an RXLR-like effector family from medically relevant fungi.

4:00 - 4:20
Yuanchao Wang
Identification and functional assay of Phytophthora sojae avirulence effectors.

4:20 - 4:40 **Break**

4:40 - 5:00
Gregory J. Fischer
Fungal lipoxygenases: a novel instigator of asthma?

5:00 - 5:20
Martha C. Giraldo
***Magnaporthe oryzae* has evolved two distinct mechanisms of effector secretion for biotrophic invasion of rice.**

5:20 - 5:40
Anupama Ghosh
Domains for plant uptake of Ustilago maydis secreted effectors.

5:40 - 6:00
Edouard Evangelisti
Penetration-specific effectors from *Phytophthora parasitica* favour plant infection.

Friday, March 15 3:00 PM–6:00 PM
Chapel

Cell Wall, Polarity and Hyphal Tip Growth

Co-chairs: Stephan Seiler and Ernestina Castro-Longoria

Abstracts for this session begin on page 67.

3:00 - 3:20
Michael Bölker
The function of Rho type small GTPases for cell polarity in *Ustilago maydis*.

3:20 - 3:40
Peter Sudbery
A quantitative model of hyphal tip growth based on the spatial distribution of exocyst subunits in the human fungal pathogen *Candida albicans*.

3:40 - 4:00
Johannes Wagener
Cell wall integrity signaling in *Aspergillus fumigatus*.

4:00 - 4:20
Roland Wedlich-Soldner
Optimization of polarity establishment through coupling of multiple feedback loops.

4:20 - 4:40 **Break**

4:40 - 5:00
Vincent Bulone
Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis.

5:00 - 5:20
Lakshmi Preethi Yerra
Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function.

5:20 - 5:40
Diego Delgado-Álvarez
Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites.

5:40 - 6:00
Norio Takeshita
Visualization of apical membrane domains in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM).

Friday, March 15 3:00 PM–6:00 PM
Heather

Sexual Regulation and Evolution in the Fungi

Co-chairs: Frances Trail and Nicolas Corradi

Abstracts for this session begin on page 69.

3:00 - 3:20
Ignazio Carbone
Clonality and sex impact aflatoxigenicity in *Aspergillus* populations.

3:20 - 3:40
Nicolas Corradi
Toolkit for sexual reproduction in the genome of Glomus spp; a supposedly ancient asexual lineage.

3:40 - 4:00
Frances Trail
Comparative transcriptomics identifies new genes for perithecium development.

4:00 - 4:20
Hanna Johannesson
Rapid evolution of female-biased genes: a novel example from the eukaryotic model organism *Neurospora crassa*.

4:20 - 4:40 **Break**

4:40 - 5:00
Katherine A. Borkovich
Self-attraction can not bypass the requirement for two mating type genes during sexual reproduction in *Neurospora crassa*.

5:00 - 5:20
Céline M. O'Gorman
Fertility in *Aspergillus fumigatus* and the identification of an additional 'supermater' pair.

5:20 - 5:40
Julia Böhm
Sexual reproduction and mating type function in the penicillin producing fungus *Penicillium chrysogenum*.

5:40 - 6:00
Patrik Inderbitzin
The *Sclerotinia sclerotiorum* mating type locus (*MAT*) contains a 3.6-kb region that is inverted in every generation.

Friday, March 15 3:00 PM–6:00 PM
Fred Farr Forum

Oxidative Stress, ROS Signaling and Adaptation to Hypoxia

Co-chairs: Geraldine Butler and Barry Scott

Abstracts for this session begin on page 72

3:00 - 3:20
A. Nantel
Transcriptional regulatory networks controlling the early hypoxic response in Candida albicans.

3:20 - 3:40
Olaf Kniemeyer
Proteomic analysis of the hypoxic response of the human-pathogenic fungus *Aspergillus fumigatus*.

3:40 - 4:00
N. Ponts
Fgap1-mediated response to oxidative stress in trichothecene-producing *Fusarium graminearum*.

4:00 - 4:20
Nallely Cano-Dominguez
The role of NADPH oxidases in *Neurospora crassa* cell fusion.

4:20 - 4:40 **Break**

4:40 - 5:00
Elizabeth A. Veal
Peroxiredoxins in ROS responses -Why evolve peroxidases that are inactivated by peroxides?

5:00 - 5:20
Lauren S. Ryder
NADPH oxidases regulate septin-mediated cytoskeletal re-modeling during plant infection by the rice blast fungus Magnaporthe oryzae.

5:20 - 5:40
Gemma M. Cartwright
Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont *Epichloë festucae*.

5:40 - 6:00
Benjamin A. Horwitz
Interaction between phenolic and oxidant signaling in *Cochliobolus heterostrophus*.

Friday, March 15 3:00 PM–6:00 PM
Kiln

Phylogenomics

Co-chairs: Jason Stajich and Joey Spatafora

Abstracts for this session begin on page 75.

3:00 - 3:20
Dannie Durand
Characterizing Gene Tree Incongruence on a Genome Scale.

3:20 - 3:40
Mary L. Berbee
Early fungi and their carbohydrate active enzymes.

3:40 - 4:00
Jason Slot
Better evolution through gene clustering.

4:00 - 4:20
C. Alisha Owensby
Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales.

4:20 - 4:40 **Break**

4:40 - 5:00
Robert Riley
Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum.

5:00 - 5:20
Helene Chiapello
Genome evolution of fungal pathogens from the Magnaporthe oryzae/grisea clade.

5:20 - 5:40
Jonathan Grandaubert
***Leptosphaeria maculans* 'brassicae': "Transposable Elements changed my life, I feel different now".**

5:40 - 6:00
Emily A. Whiston
Comparing comparative "omics" in *Coccidioides spp*.

Friday, March 15 3:00 PM–6:00 PM
Nautilus

Synthetic Biology

Co-chairs: Nancy Keller and Peter Punt

Abstracts for this session begin on page 78.

3:00 - 3:20
Debbie S. Yaver
Engineering Aspergillus oryzae for high level production of L-malic acid.

3:20 - 3:40
Pascale Daran-Lapujade
When synthetic biology meets metabolic engineering: *in vivo* pathway assembly in *Saccharomyces cerevisiae*.

3:40 - 4:00
Levente Karaffa
Analysis of the intracellular galactoglycom of Trichoderma reesei grown on lactose.

4:00 - 4:20
Peter J. Punt
Novel transcriptomics approaches for metabolic pathway engineering target identification in *Aspergillus*.

4:20 - 4:40 **Break**

4:40 - 5:00
Y. Huang
A new method for gene mining and enzyme discovery.

5:00 - 5:20
Koichi Tamano
Increased production of fatty acids and triglycerides in *Aspergillus oryzae* by modifying fatty acid metabolism.

5:20 - 5:40
Ana Rems
Molecular biological basis for statin resistance in naturally statin-producing organisms.

5:40 - 6:00
Hong Luo
Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms.

CONCURRENT SESSIONS SCHEDULES

Friday, March 15 3:00 PM–6:00 PM
Scripps

Fungicides and Antifungal Compounds

Co-chairs: Daniele Debieu and Paul Verweij

Abstracts for this session begin on page 81.

3:00 - 3:20
D. A. Macdonald
Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*.

3:20 - 3:40
Branka Korosec
Inhibition of benzoate 4-monooxygenase (CYP53A15) from *Cohliobolus lunatus* by cinnamic acid derivatives.

3:40 - 4:00
Marcelo HS Ramada
Secretome analysis of *Trichoderma harzianum* cultivated in the presence of *Fusarium solani* cell wall or glucose.

4:00 - 4:20
Carol E. Davis
Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers.

4:20 - 4:40 **Break**

4:40 - 5:00
Paul E. Verweij
The fungi strike back: multidrug resistance in Aspergillus fumigatus and agricultural use of fungicides.

5:00 - 5:20
D. Sanglard
Effect of antifungal resistance on virulence of *Candida* spp.

5:20 - 5:40
Sabine Fillinger
From enzyme to fungal development or how *sdhB* mutations impact respiration, fungicide resistance and fitness in the grey mold agent *Botrytis cinerea*.

5:40 - 6:00
Gabriel Scalliet
Deciphering fungicide resistance mechanisms in phytopatogenic fungi, towards an assessment of resistance risk in new active ingredient research.

Saturday, March 16 2:00 PM–5:00 PM
Merrill Hall

Parallels between Fungal Pathogens of Plants and Animals

Co-chairs: Barbara Howlett and Axel Brakhage

Abstracts for this session begin on page 84.

2:00 - 2:20
Sarah J. Gurr
Emerging fungal (and Oomycete) threats to plant and ecosystem health.

2:20 - 2:40
Axel A. Brakhage
Melanin as virulence determinant of human and plant pathogenic fungi.

2:40 - 3:00
Joanna Potrykus
Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections.

3:00 - 3:20
A. Sharon
Common strategies in plant and human "necrotrophic" pathogens: role of PCD.

3:20 - 3:40 **Break**

3:40 - 4:00
Nick J. Talbot
Septin-mediated plant tissue invasion by the rice blast fungus Magnaporthe oryzae.

4:00 - 4:20
Katja Schaefer
Components of the urease complex govern virulence of *Fusarium oxysporum* on plant and animal hosts.

4:20 - 4:40
Anja Kombrink
The role of LysM effectors in fungal fitness.

4:40 - 5:00
Harshini C. Weerasinghe
Genes important for *in vivo* survival of the human pathogen Penicillium marneffei.

Saturday, March 16 2:00 PM–5:00 PM
Chapel

Secondary Metabolism

Co-chairs: Gillian Turgeon and Bettina Tudzynski

Abstracts for this session begin on page 87.

2:00 - 2:20
B. Condon
Genomic profiles of secondary metabolism genes in *Cochliobolus* pathogens.

2:20 - 2:40
Candace Elliott
A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, *Leptosphaeria maculans*.

2:40 - 3:00
Eva-Maria Niehaus
Fusarin C biosynthesis in *Fusarium fujikuroi*: the fusarin C gene cluster, their function and regulation.

3:00 - 3:20
H. Corby Kistler
Cellular development integrating primary and induced secondary metabolism in the filamentous fungus *Fusarium graminearum*.

3:20 - 3:40 **Break**

3:40 - 4:00
Nancy Keller
LaeA sleuthing reveals cryptic gene clusters in pathogenic Aspergilli.

4:00 - 4:20
Kristina M. Smith
The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in *Fusarium graminearum*.

4:20 - 4:40
M. Viaud
Secondary metabolism in *Botrytis cinerea*: the grey and pink sides of a pathogen.

4:40 - 5:00
Frank Kempken
Is fungal secondary metabolism regulated by competing insects?

Saturday, March 16 2:00 PM–5:00 PM
Heather

Light Sensing and Circadian Rhythms

Co-chairs: Luis Larrondo and Reinhard Fischer

Abstracts for this session begin on page 90.

2:00 - 2:20
Martha W. Merrow
Circadian rhythms in gene expression in Aspergillus nidulans.

2:20 - 2:40
C. Hong
Circadian clock-gated cell division cycles in *Neurospora crassa*.

2:40 - 3:00
Kevin K. Fuller
Light regulates growth, stress resistance and metabolism in the fungal pathogen *Aspergillus fumigatus*.

3:00 - 3:20
Paulo Canessa
Shedding light on Botrytis biology: characterization of the WC1 photoreceptor and FRQ homologues in the necrotrophic plant pathogen *Botrytis cinerea*.

3:20 - 3:40 **Break**

3:40 - 4:00
Carmen Ruger-Herreros
The transcription factor FL is phosphorylated and interacts with a trehalose related protein in *Neurospora crassa*.

4:00 - 4:20
Alfredo H. Herrera-Estrella
Regulation of gene expression in response to light in *Trichoderma atroviride*.

4:20 - 4:40
Victoriano Garre
Genome-wide analysis of light responses in *Mucor circinelloides*.

4:40 - 5:00
Phillipp Wiemann
Shedding light on secondary metabolite cluster gene expression, sporulation, UV-damage repair and carotenogenesis in the rice pathogen *Fusarium fujikuroi*.

Saturday, March 16 2:00 PM–5:00 PM
Fred Farr Forum

Fungal Evo-Devo

Co-chairs: Steve Harris and Brian Shaw

Abstracts for this session begin on page 93.

2:00 - 2:20
Antonis Rokas
The Molecular Foundations of the Fungal Lifestyle.

2:20 - 2:40
Daniel J. Ebbole
Gene expression and regulation during conidial morphogenesis in *Neurospora crassa*.

2:40 - 3:00
David S. Hibbett
Comparative developmental morphology in lentinoid mushrooms: toward a new fungal evo-devo?

3:00 - 3:20
Steven D. Harris
The Cdc42 GTPase module and the evolution of conidiophore architecture in *Aspergillus*.

3:20 - 3:40 **Break**

3:40 - 4:00
Audrey M. V. Ah-Fong
Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase.

4:00 - 4:20
Jurgen W. Wendland
Molecular Determinants of Sporulation in *Ashbya gossypii*.

4:20 - 4:40
Heesoo Park
THE *velvet* regulators in *Aspergilli*.

4:40 - 5:00
R. Debuchy
A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*.

Saturday, March 16 2:00 PM–5:00 PM
Kiln

Environmental Metagenomics

Co-chairs: Chris Schadt and Betsy Arnold

Abstracts for this session begin on page 95.

2:00 - 2:20
Donald R. Zak
Microbial Responses to a Changing Climate: Implications for the Future Functioning of Terrestrial Ecosystems.

2:20 - 2:40
Mizue Naito
The Interaction of *Mycoplasma*-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host.

2:40 - 3:00
Ning Zhang
Metagenomic analysis reveals hidden fungal diversity in grass rhizosphere and tree foliage.

3:00 - 3:20
Weiguo Fang
Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus.

3:20 - 3:40 **Break**

3:40 - 4:00
Kabir Peay
Structure and function of soil fungal communities across North American pine forests.

4:00 - 4:20
Gregory Bonito
Genomic analysis of Mortierella elongata and its endosymbiotic bacterium.

4:20 - 4:40
Richard C. Hamelin
Integrative genomics of poplar-fungal pathogen interactions.

4:40 - 5:00
M.-S. Benitez
Fungal pathogen and endophyte genetics within the context of forest community dynamics.

Saturday, March 16 2:00 PM–5:00 PM
Nautilus

Dimorphic Transitions

Co-chairs: Anne Dranginis and Alex Andrianopoulos

Abstracts for this session begin on page 97.

2:00 - 2:20
Richard Bennett
Epigenetic Switching Regulates the Yeast-Hyphal Transition in *Candida albicans*.

2:20 - 2:40
Linqi Wang
Extracellular and intracellular signaling orchestrates morphotype-transition and virulence in human pathogen *Cryptococcus neoformans*.

2:40 - 3:00
Chad A. Rappleye
***Histoplasma* strain variations and differences in pathogenic-phase transcriptomes.**

3:00 - 3:20
Hayley E. Bugeja
The C_2H_2 transcription factor HgrA promotes hyphal growth in the dimorphic pathogen *Penicillium marneffei*.

3:20 - 3:40 **Break**

3:40 - 4:00
Joerg T. Kaemper
A conserved splicing factor is required for vesicle transport in *Ustilago maydis*.

4:00 - 4:20
Sarah A. Gilmore
***N*-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi.**

4:20 - 4:40
Gregory M. Gauthier
A GATA transcription factor encoded by *SREB* functions as a global regulator of transcription in *Blastomyces dermatitidis.*

4:40 - 5:00
Bridget M. Barker
Functional Analysis of Genes in Regions of Introgression in Coccidioides.

Saturday, March 16 2:00 PM–5:00 PM
Scripps

Tropic Growth and Fusion

Co-chairs: Andre Fleissner and Nick Read

Abstracts for this session begin on page 100.

2:00 - 2:20
Carla J. Eaton
Role of the cell fusion gene *idcA* in fungal mutualism.

2:20 - 2:40
Pablo S. Aguilar
Role of extracellular calcium in budding yeast cell fusion.

2:40 - 3:00
Chia-Chen Chang
The role of calcium and calmodulin during cell fusion and colony initiation in *Neurospora crassa*.

3:00 - 3:20
Javier Palma-Guerrero
LFD-1 is a component of the membrane merger machinery during cell-cell fusion in *Neurospora crassa*.

3:20 - 3:40 **Break**

3:40 - 4:00
Martin Weichert
Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in *Neurospora crassa*.

4:00 - 4:20
David Turra
Co-option of a sex pheromone receptor and MAPK signalling pathway for chemotropism of Fusarium oxysporum towards plant host compounds.

4:20 - 4:40
Britta Herzog
Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete *Sordaria macrospora*.

4:40 - 5:00
Darren Thomson
Characterisation of contact-dependant tip re-orientation in *Candida albicans* hyphae.

PLENARY SESSION ABSTRACTS

Wednesday, March 13 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session I: Metabolic Pathways: Cell Growth, Pathogenesis and Bioenergy

Chair: Louise Glass

Hypoxia and Mechanisms of Human Fungal Pathogenesis: To Air or Not to Air? Robert A. Cramer. Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Human disease caused by fungi is increasing in frequency and clinical outcomes remain unacceptably poor due in part to a limited spectrum of treatment options. New insights into fungal pathogenesis mechanisms has great potential to uncover new therapeutic options for these devastating diseases. Our laboratory is investigating how the most common causal agent of human airborne fungal infections, Aspergillus fumigatus, adapts and grows in in vivo microenvironments generated during the fungal-host interaction. We have observed that significant oxygen depletion, hypoxia, occurs at sites of A. fumigatus infection in the lung. The ramifications of hypoxia on obligate aerobic fungal metabolism, both from the perspective of in vivo fungal growth and the production of fungal metabolites that influence the innate immune response, are largely unknown. Using a combination of genomics, fungal molecular genetics, animal models of fungal disease, and immunology we have begun to unravel the impact of hypoxia on outcomes of invasive pulmonary aspergillosis. We have observed that fungal metabolic responses to hypoxia are largely interconnected with an increased demand for iron uptake that directly influences metabolic pathways requiring both oxygen and iron as co-factors such as ergosterol, heme, and cell wall biosynthesis. These responses are regulated in part by 2 transcription factors with sequence similarity to the sterol regulatory element binding protein family (SREBPs). In turn, these metabolic pathways are not only essential for fungal growth under hypoxia, but also for the production of pathogen associated molecular patterns (PAMPs) that influence the innate immune response to the invading fungus. Our data suggest that hypoxia alters the composition of the fungal cell wall resulting in increased production of pro-inflammatory cytokines from host effector cells that may cause host tissue damage. Taken together, our data suggest that manipulation of in vivo oxygen levels may be a promising strategy to augment existing antifungal drug treatment through manipulation of fungal metabolism and improve patient outcomes from these increasingly common human diseases.

Tackling biofuel bottlenecks through genome wide association studies in *Saccharomyces cerevisiae*. Dana J. Wohlbach[1,2], Trey Sato[1], Audrey P. Gasch[1,2]. 1) Great Lakes Bioenergy Research Center, Univ Wisconsin, Madison, Madison, Wi; 2) Laboratory of Genetics, Univ Wisconsin, Madison, Madison, WI.

Generating biofuels from cellulosic plant material is a major goal in bioenergy research. However, a critical bottleneck is the inhibition of microbial fermentation by toxic compounds in the hydrolyzed plant biomass, generated during chemical pretreatment. We are exploiting natural variation in yeast hydrolysate tolerance to implicate genes and processes for targeted strain engineering. We phenotyped growth rates of 65 diverse wild and industrial *S. cerevisiae* strains grown in several different plant hydrolysates, and then performed a genome-wide association study (GWAS) to identify loci that correlated with hydrolysate tolerance. This identified nearly 70 loci, encompassing over 100 genes that fell into specific pathways and signaling networks. We also identified pervasive epistasis across loci, suggesting the importance of gene-gene interactions in phenotypic variation. The results provide a platform for synthetic engineering of hydrolysate tolerance in diverse yeast strains.

Exploring the metabolome of cereal infecting fusaria. Kim E. Hammond-Kosack[1], Rohan G.T. Lowe[1], Gail Canning[1], Martin Urban[1], Michael H. Beale[2], Jane L. Ward[2]. 1) Dept of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; 2) National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK.

The Ascomycete fungus *Fusarium graminearum* (*Gibberella zeae*) causes plant disease on many cereal crop species including wheat, barley and maize and is also becoming a problem pathogen in several non-cereal crop species, including soybean and sugar beet. In cereal crops, floral infections at anthesis go on to reduce grain yield and quality and contaminate the crop with secondary metabolites harmful to plants, animals and humans. These metabolites include trichothecene mycotoxins such as deoxynivalenol (DON) and their acetylated derivatives. We have already reported on the use of metabolomic analysis to understand the basal metabolism in four Fusarium spp., *F. graminearum*, *F. culmorum*, *F. pseudograminearum* and *F. venenatum* under DON and non-DON inducing conditions (Lowe et al., 2010, MPMI, 23, 1605-1618). Three additional studies have now been completed using a 'triple-fingerprint' of analytical techniques including ^{1}H-NMR and electrospray mass-spectroscopy (+/- ESI-MS). First, we have investigated the global metabolic changes occurring during a time course of DON mycotoxin induction *in vitro* using the *F. graminearum* wild-type strain PH-1 for which the complete genomic sequence is available (http://www.broad.edu; www.phytopathdb.org). Second, we have characterised many single gene deletion mutants affected in mycotoxin biosynthesis, cell signalling and plant pathogenicity. Interestingly, most mutants show significant and highly specific metabolome changes compared to the parental wild-type strain(s). Third, we have explored the metabolome of several reduced virulence mutants arising from a random plasmid insertion, forward genetic screen to determine whether the triple fingerprinting technique can be used predictively. Metabolic network analysis of the data sets is now being used to link the co-occurrence of known and unknown metabolites to DON mycotoxin production and to aid the future characterisation of the many unknown metabolites present in *F. graminearum*. This research was supported by a metabolomics special initiative grant from the Biotechnology and Biological Sciences Research Council.

Carbohydrate-active enzymes in fungal genomes. Bernard Henrissat. AFMB, CNRS and Aix-Marseille University, Marseille, France.

We term carbohydrate-active enzymes (CAZymes) the enzymes that assemble and breakdown complex carbohydrates and carbohydrate polymers. As such carbohydrates are crucial for fungi as carbon sources but also for cell wall synthesis/remodelling, host pathogen interactions, energy storage etc. Unlike many other classes of enzymes which carry limited informative power, the peculiarities of CAZymes and of their substrates turn these enzymes into extremely powerful probes to examine genomes and explain the lifestyle of living organisms and fungi in particular. Over the last few years we have explored the CAZyme content of over 200 fungal genomes and we will review how evolution shapes the CAZyme profiles of fungi.

Suggested reading :

- Cantarel et al. (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res. 37: D233-D238
- Ohm et al. (2012) Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. PLoS Pathogens, 8(12): e1003037.
- O'Connell et al.(2012) Life-style transitions in plant pathogenic Colletotrichum fungi defined by genome and transcriptome analyses. Nature Genetics 44, 1060-1065
- Floudas et al. (2012) The Paleozoic origin of white rot wood decay reconstructed using 31 fungal genomes. Science, 336, 1715-1719
- Ma et al. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium oxysporum*. Nature 464, 367-373
- Martin et al. (2010) Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464, 1033-1038.

Genome-wide approaches to identify and characterize lignocellulolytic enzymes. Adrian Tsang. Biol, Concordia Univ, Montreal, Canada.

Lignocellulosic material is both the most abundant source of biomass on the planet and an enormous storehouse of sugars. Yet the sugars in cellulosic material are remarkably recalcitrant. The ability to detect new enzymes, to produce them in large quantities, and to understand how they work will lay the groundwork for the development of more efficient and economical processes for lignocellulosic biomass. We are particularly interested in harnessing the ligocellulolytyic ability of thermophilic fungi as they are potential reservoirs of thermostable enzymes for industrial applications. So far, fewer than 50 fungal species have been described as thermophiles. We have sequenced over 20 species of thermophilic fungi, see www.fungalgenomics.ca. Most of these thermophiles belong to the orders Sordariales and Eurotiales, three species belong to the Mucorales and one to Onygenales. We have developed computational tools to improve the identification genes in fungal genomes in general, and genes encoding extracellular proteins in particular because biomass-degrading enzymes are predominantly extracellular proteins. In addition to using informatics tools to identify orthologues of lignocellulolytic enzymes, we have analyzed the transcriptomes and exo-proteomes of the thermophilic fungi when cultured in a variety of agricultural straws to reveal the strategies used by different fungi in the decomposition of lignocellulose as well as identifying novel extracellular proteins that may play a role in biomass decomposition. Over 2000 genes encoding potential lignocellulolytic proteins have been identified. The Sordariales possess a larger repertoire of lignocellulolytic enzymes than the thermophiles from other orders. The genes predicted to encode lignocellulolytic proteins have been cloned and transformed into Aspergillus niger for the production of recombinant enzymes. Biochemical characterization of the recombinant enzymes show that in addition to producing enzymes that are thermostable, the thermophiles also produce enzymes that have temperature optimum in the 40-50°C range.

Thursday, March 14 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session II: Organismic Molecular Interactions

Chair: Nick Talbot

Large-scale Biology for Fungal Pathogenicity in *Magnaporthe oryzae*. Yong-Hwan Lee[1,2]. 1) Department of Agricultural Biotechnology, Seoul National Univ, Seoul, 151-921, Korea; 2) Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea.

Rice blast is a compelling model system for studying host-parasite interactions due to its socioeconomic impact and the availability of both the rice and fungal genomic sequences. In an attempt to understand the molecular mechanisms of rice blast, we have been taking both forward and reverse genetics approaches. Our researches using reverse genetics approach focus on identifying and characterizing the genes involved in signal transduction pathways leading to appressorium formation, genes encoding transcription factors, and genes that are required for post-penetration stages. For forward genetics studies, we carried out a large-scale insertional mutagenesis of the *Magnaporthe oryzae* strain KJ201 via *Agrobacterium tumefaciens*-mediated transformation, generating over 25,000 mutants. We also developed high throughput phenotype screening system that enables rapid and robust assay of mutant phenotypes. In addition to our endeavor to functional and comparative genomics, we built a cyber-infrastructure for storage of heterogeneous data and analysis of such data in multiple contexts. The whole genome sequence information of *M. oryzae* as well as most of the results from experimental biology is housed in our customized database. Our comprehensive and integrative approaches coupled with a web-based Laboratory Information Management System would provide a novel platform for systems biology initiatives for fungal pathogenesis.

MAPK-mediated control of infectious growth in *Fusarium oxysporum*. Antonio Di Pietro. Departamento de Genetica, Universidad de Cordoba, 14071 Cordoba, Spain.

In fungal pathogens, contact with the host triggers a developmental and metabolic transition towards infectious growth. What exactly defines infectious growth and how it is controlled by environmental and host-derived stimuli is not fully understood. We study infectious growth in *Fusarium oxysporum*, a soilborne fungus that causes vascular wilt disease on a wide range of plant species and opportunistic infections in immunocompromised humans. One of the key players in pathogenicity is Fmk1, a conserved mitogen-activated protein kinase (MAPK) that is essential for infection-related processes such as chemotropism, host adhesion, penetration and invasive growth in the plant tissue. Most Fmk1-dependent virulence functions require the homeodomain transcription factor Ste12, and are repressed in the presence of the preferred nitrogen source ammonium through a mechanism that requires the transporter MepB and the bZIP factor MeaB. Recent data suggest that ammonium repression is mediated by a shift in extracellular pH, which results in rapid changes in the phosphorylation pattern of different MAPKs. Our current research addresses the mechanisms through which pH controls invasive growth of *F. oxysporum* by reprogramming the activation status of cellular MAPK signalling cascades.

Analysis of effector proteins from flax rust and wheat stem rust. Peter Dodds[1], narayana Upadhyaya[1], Ann-Maree Catanzariti[2], Markus Koeck[1], Adnane nemri[1], Rohit Mago[1], Simon Williams[3], Thomas Ve[3], Maryam Rafiqi[4], Wenjie Wu[2], Adrienne Hardham[2], David Jones[2], Jeff Ellis[1], Bostjan Kobe[3], Robert Park[5]. 1) Plant industry, CSIRO, Canberra, ACT, Australia; 2) Australian National University, Research School of Biology,; 3) University of Queensland, School of Chemistry and Molecular Biosciences; 4) Justus Liebig University, Giessen, Germany; 5) University of Sydney, Camden.

Rust fungi cause economically important diseases of cereal crops worldwide, with stem rust caused by the fungus Puccinia graminis tritici one of the most serious diseases in wheat. Because of the ability of the fungus to evolve increased virulence towards previously resistant varieties, continuous breeding and the identification of new sources of resistance is necessary to keep apace of the threat of rust epidemics. We have been studying how the plant immune system can recognise and respond to rust pathogens using the flax rust model system. Rusts are obligate parasites of plants, and produce a specialised infection structure called the haustorium which directly penetrates an infected cell and is the main site of nutrient extraction for the fungus. A suite of disease effector proteins are secreted from haustoria and enter the host cells where they may allow the rust to commandeer host cell biology. It is these translocated effector proteins that are recognised by host immune receptors, known as resistance (R) proteins. We have been exploring the structure and function of host-translocated effectors from flax rust and also searching for effector candidates from stem rust that are recgonised by known wheat R genes.Using genome and transcriptome sequencing we have predicted, carefully curated and analysed the transcription of 400 candidate effector genes from the Australian stem rust strain 21-0. To screen for effectors recognized by wheat R genes, we have developed a bacterial Type III Secretion System (TTSS)-based delivery assay from the non-pathogen Pseudomonas fluorescens strain Pfo. We are screening candidate effectors on a set of 18 wheat cultivars carrying 22 different R genes. Thus far we have identified on effector protein induces a rapid cell death response specifically on a wheat genotype carrying Sr22. We are also analyzing sequence variation in effector candidates between clonal field isolates that have mutated to overcome the resistance genes that have been deployed in agriculture.

PLENARY SESSION ABSTRACTS

Dissecting *Phytophthora* blight; making sense out of signalling, effectors and host targets. Francine Govers. Lab. of Phytopathology, Wageningen University, Wageningen, Netherlands.

The plant pathogen *Phytophthora infestans* causes late blight, the disease that was responsible for the Irish potato famine in the mid-nineteenth century. This oomycete has a hemibiotrophic life style, a narrow host range and a large genome of ~ 240 Mb. Comparative genomics revealed features illuminating its success as a pathogen, such as rapid turnover and massive expansion of families encoding secreted proteins, and peculiar gene innovations resulting in proteins with oomycete-specific domain combinations. An example of a novel protein family is the GPCR-PIPK family. Its twelve members all have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with a phosphatidylinositol phosphate kinase (PIPK) domain at the C-terminus. This domain structure suggests that GPCR-PIPKs use GPCRs to directly feed extracellular signals into phospholipid signalling pathways. Their differential expression and localization point to distinct roles in various cellular processes. For one GPCR-PIPK we could demonstrate a role in asexual development, including spore germination, hyphal elongation and sporangia cleavage, whereas inactivation of another GPCR-PIPK disturbs sexual development. For successful infection *Phytophthora* secretes a variety of proteins including a large number of effectors that share the host-cell targeting motif RXLR. Inside host cells these RXLR effectors promote virulence by manipulating the cell machinery via interaction with host targets thereby suppressing host defence. However, in plants carrying matching resistance genes RXLR effectors trigger defence and thus act as avirulence factors. Here I will focus on an RXLR effector that interacts with an exocyst component and show how the interplay between this effector and its host target influences the host-pathogen interaction.

Understanding directional growth in fungi. Alexandra C. Brand. Aberdeen Fungal Group, Univ Aberdeen, Scotland, United Kingdom.

Fungal hyphae are programmed to explore their surroundings in search of nutrients and, for pathogens, success can depend on locating and identifying suitable host penetration sites. Fungi have therefore evolved mechanisms that link the sensing of environmental cues with an appropriate growth response. The intracellular components involved in polarised growth in fungi are generally well-conserved and have been studied in model organisms such as *Saccharomyces cerevisiae*, *Neurospora crassa* and *Aspergillus* spp. However, how environmental signals interact with the molecular machinery of hyphal tip growth is less well-understood. *Candida albicans* is an opportunistic pathogen that exhibits pre-programmed, or tropic, growth responses to specific stimuli. This makes it a useful model for dissection of the regulatory pathways that control hyphal tip behaviour. A variety of external stimuli, including electric fields, surface modification and nanofabrication techniques, have been used to examine the physical properties of apical growth, such as directional memory, asymmetric tip organisation and hyphal tip force. In addition, these methods have been coupled with reverse genetics, fluorescence protein-tagging and live-cell imaging to identify cell-polarity components that can enhance, or even reverse, the direction of hyphal growth. The evidence to date suggests that the direction of hyphal growth reflects the net output from overlapping positional determinants. In addition, there is a strong association between proper hyphal tip regulation and the ability of a fungus to invade and damage host tissue.

Friday, March 15 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session III: Sensing, Cell Biology and Development

Chair: Michelle Momany

The illuminated Spitzenkörper of *Neurospora crassa*: tracking and tracing secretory vesicles. Meritxell Riquelme[1], Eddy Sánchez-León[1], Rosa Fajardo-Somera[1], Erin L. Bredeweg[2], Olga Callejas-Negrete[1], Robert W. Roberson[3], Salomon Bartnicki-García[1], Michael Freitag[2]. 1) Dept Microbiology, Center for Scientific Research and Higher Education of Ensenada CICESE, Ensenada, Baja California 22860, Mexico; 2) Center for Genome Research and Biocomputing, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A; 3) School of Life Sciences,Arizona State University, Tempe, AZ 85287, U.S.A.

Tip growth in fungal hyphae is maintained by the vectorial traffic of secretory vesicles to the apex, where they accumulate at the Spitzenkörper (Spk), before fusing with the apical plasma membrane (PM) to provide the enzymatic machinery necessary for cell expansion. Confocal microscopy of *Neurospora crassa* strains expressing fluorescently tagged proteins that are predicted to participate in cell wall synthesis revealed that the Spk vesicles contain different enzymatic activities. Microvesicles (chitosomes) at the core of the Spk contained chitin synthases CHS-1, -3, -4, -5 and -6, whereas macrovesicles at the outer layer carried glucan synthase (GS). The coordinated action of coats, tethers, motors, Rabs and SNAREs allows the multiple vesicular carriers and their cargoes to traffic between organelles and to be delivered at their final destinations. While it remains to be elucidated what regulates the spatial stratification of the Spk, our most recent analyses show the differential co-localization of Rab GTPases YPT-1 and SEC-4 with micro and macrovesicles, respectively; suggesting different biogenesis for these vesicles. Prior to v-SNARE and t-SNARE recognition and fusion with the PM, secretory vesicles are presumably tethered to their target acceptor membrane in a process mediated by the exocyst, an octameric complex. In *N. crassa* an intact exocyst complex is required for formation of the Spk and the maintenance of regular hyphal growth. Two distinct localization patterns of the exocyst subunits were observed at the hyphal tip. EXO-70 and EXO-84 accumulated at the frontal part of the Spk external layer, coinciding partially with the macrovesicular layer, whereas the exocyst components SEC-3, 5, 6, 8 and 15 formed a delimited crescent at the apical PM. This suggests the formation of two distinct exocyst subcomplexes that may unite during vesicle tethering in post-Spk traffic steps. Collectively our results prove the direct involvement of the Spk in cell wall synthesis and confirm that the region of exocyst-mediated vesicle fusion in the hyphal apex coincides with the exocytotic gradient predicted by the Vesicle Supply Center (VSC) model for fungal morphogenesis, with a maximum at the pole and vanishing gradually in the subapex.

Evolution of sexual reproduction: A view from the Fungal Kingdom. Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University, heitm001@duke.edu.

Sex is nearly universal in eukaryotes, and thought to have evolved once. Sex promotes genetic diversity and evolution, yet also confers costs. Both mechanisms of sex determination and mechanics of sexual reproduction are remarkably diverse. Fungi are exceptional models to analyze these processes, and their study reveals surprising insight into both sex and its impact. We focus on how mating-type identity is specified and modes and roles of sexual reproduction in generating diversity. Many fungi have bipolar sexual cycles with two opposite mating types and a bi-allelic mating type locus. In the Basidiomycota many species have a more complex tetrapolar sexual cycle with two unlinked multi-allelic mating type loci, resulting in thousands of mating types and enhanced outcrossing but restricted inbreeding. Our studies reveal how transitions from ancestral tetrapolar to derived bipolar systems have occurred in pathogenic species embedded within saprobic sibling taxa. The tetrapolar-bipolar transition has occurred repeatedly in pathogens of plants and animals, suggesting it might be selected during host adaptation. Pathogenic *Cryptococcus* species have taken this transition further to a unipolar sexual cycle. These species have global largely unisexual populations and reproduce via an unusual homothallic unipolar sexual cycle involving only one mating type (same-sex mating, unisexual reproduction). Like **a**-a opposite sex mating, a-a unisexual mating can admix parental diversity in the progeny. However, in other cases solo a-a unisex involves selfing of identical genomes with no genetic diversity to exchange. Why organisms engage in selfing challenges conventional views on the roles of sex. We find unisex generates genetic diversity de novo, preserving well-adapted genomic configurations while generating more limited genetic diversity for selection to act upon. Discovery that other fungi and eukaryotic parasite pathogens also reproduce unisexually generalizes these findings, and suggests unisex may have evolved because it mitigates costs of sex. Studies of fungal sex and its evolution and impact illustrate general principles by which diversity is generated and maintained with implications for saprobic and pathogenic microbes and multicellular eukaryotes.

Metabolic compensation of the Neurospora clock by a glucose-dependent feedback of the circadian repressor CSP1 on the core oscillator. Gencer Sancar, Cigdem Sancar, Francois Cesbron, Michael Brunner. Dpet Biochemistry, Univ Heidelberg, Heidelberg, Germany.

CSP1 is a global morning specific transcription repressor of Neurospora that modulates expression of about 800 genes. Expression of CSP1 is stimulated by glucose and under circadian control of the white-collar complex (WCC). In csp1 mutant strains the circadian period length decreases with increasing glucose concentrations due to increased expression of WC1. In contrast, in wild-type strains the period is compensated for changes in glucose concentration and WC1 levels are independent of glucose. CSP1 contributes to metabolic compensation of the circadian clock by glucose-dependent repression of wc1 transcription, which counterbalances the glucose-dependent translation efficiency of wc1 RNA. Forced over-expression of CSP1 reduces of WC1 expression and results in dampening of the circadian clock. Many target genes of CSP1 are rhythmically expressed with an evening specific phase while target genes of the WCC are morning specific.

Integration of the fungal cell cycle with growth and development. Meera Govindaraghavan, Kuo-Fang Shen, Stephen Osmani. Dept Molec Gen, Ohio State Univ, Columbus, OH.

A universally important aspect of growth and development is the integration of mitosis with cell division. This helps ensure that cells maintain their normal size, shape and nuclear number, which in the fungi can vary considerably. For example, the highly polarized mode of growth of the filamentous fungi is subject to complex developmental regulation yielding diverse cell types containing from one to dozens of nuclei. How fungi integrate the regulation of the developmental axis involving mitosis, cytokinesis, and morphogenesis to maintain their defined cellular shapes, with distinctive numbers

of nuclei, remains a mystery. However, recent studies of the mitotic NIMA kinase indicates it plays additional non-mitotic cytoplasmic functions in *Aspergillus nidulans* that impinge on fungal development. These insights were derived initially from defining the interphase subcellular locations of NIMA which revealed it locates to both forming and mature septa and additionally locates to tips of growing interphase cells. Subsequent studies revealed that septal pores are subject to cell cycle regulation which prevents cytoplasmic movement between mitotic cell compartments and their adjacent interphase partners. We further find that NIMA markedly affects the regulation of cell tip dominance and morphology via a mechanism involving NIMA location at microtubule +ends and the modulation of interphase cytoskeletal functions. Collectively the findings indicate that the mitotic NIMA kinase has roles to regulate communication between adjacent hyphal cells as well as cytoskeletal functions important for normal tip cell growth. Thus NIMA has the potential to help integrate nuclear division with cell division and morphogenesis.

A Neurospora cell-free system reconstitutes peroxisome membrane protein synthesis and organelle-specific targeting. Gregory Jedd. Temasek Life Sciences Laboratory, Singapore, Singapore.

A central problem faced by eukaryotic cells is how to ensure that membrane proteins are localized to the appropriate organelle. Peroxisomes are ubiquitous eukaryotic organelles that proliferate through growth and division, and can also arise de novo from endoplasmic reticulum (ER)-derived precursors. Two distinct views for the biogenesis of peroxisome membrane proteins (PMPs) are currently entertained. In the direct targeting model, PEX19 recognizes PMPs in the cytosol and ferries them to the peroxisome where interaction with PEX3 prompts PMP release and membrane integration. In the second model, nascent PMPs are integrated to the ER membrane first, and then traffic to the peroxisome membrane. In this case, PEX19 functions as a sorting receptor to package PMPs into ER-derived vesicles. My talk will focus on development of a cell-free system that reconstitutes PMP synthesis and targeting to the peroxisome membrane. I will discuss how distinct chaperones and sequences associated with transmembrane domains distinguish direct ER and peroxisome targeting pathways.

Saturday, March 16 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session IV: Functional Ecology and Fungal Communities

Chair: Jim Anderson

Mechanisms allowing the formation of new fungal pathogenic species on novel hosts, causing emerging diseases. Tatiana Giraud. ESE, Univ Paris 11, Orsay, France.

We have studied different mechanisms allowing the formation of novel fungal pathogenic species on novel hosts. A theoretical model combined with a literature survey have confirmed that mating within hosts in pathogenic ascomycetes may allow the rapid formation of new species by host adaptation alone, without requiring the evolution of mate choice. We also present studies on mechanisms of speciation in Microbotryum a fungal complex causing the sterilizing anther-smut disease in many species of Caryophyllaceae. Multiple gene phylogenies and measures of hybrid inviability and sterility have revealed the existence of multiple cryptic species, each being specific to one or a few plant host. We investigated the evolution of reproductive isolation in the species complex, and showed that hybrid inviability and sterility increased with the genetic distance between the species. We show that selfing is the main barrier to gene flow between close species and that hybrid sterility arise because of karyotypic rearrangements. Cophylogeny analyses showed that Microbotryum species have evolved through frequent host shifts to moderately distant hosts. Current geographic distribution of host species seemed to be of little relevance for understanding the putative historical host shifts, because most fungal clades had overlapping ranges. We did detect some ecological similarities between host species that were diseased by closely related anther smut species, including pollinator or habitat similarities. However, genetics underlying the host-parasite interactions appeared to be the most important factor influencing host-shifts and specialization: multi-host species parasitized closely related plant species and related species in the Microbotryum phylogeny were associated with members of the same host clade. We performed a cross-inoculation experiment and showed that both host and pathogen phylogenies were indeed significant predictors of host range, with at least partly independent effects. We investigated whether some Microbotryum species have arisen via hybridization.We also detected hybrids in nature and underwent a population genomic study to unravel the genomic architecture of introgression. Anther smut fungi appear as excellent models to unravel the mechanisms of formation of new fungal species onto novel hosts.

The decisive role of mycorrhizal fungi as regulators of carbon sequestration in boreal forest ecosystems. B.D. Lindahl, K.E. Clemmensen, I.T.M. Bödeker, E. Sterkenburg. Dept. Forest Mycol. & Path., SLU, Uppsala, Sweden.

Boreal forest soils represent a significant global sink for carbon, but poor knowledge about the mechanisms that regulate the dynamics of soil carbon pools hampers the development of predictive ecosystem models. Such models are urgently needed to guide proper management of forest land, in order to mitigate increasing atmospheric CO2 levels. By analysing the natural abundance of rare isotopes (^{14}C, ^{13}C and ^{15}N), we found that the mycelium of mycorrhizal fungi represents a major source of soil carbon. Up to 50-70% of stored carbon was estimated to enter the soil via plant roots. Ratios between fungal biochemical markers (ergosterol and chitin) indicate that a rapid turnover of fungal mycelium minimizes carbon storage and favours efficient nitrogen recycling, whereas slow mycelial turnover favours carbon sequestration and immobilisation of nitrogen. In order to relate taxonomic and functional diversity to carbon dynamics, we used 454-sequencing of ITS2 amplicons to analyse fungal communities in environmental samples. In forest soils with low nitrogen availability we found high abundance of ectomycorrhizal genera with differentiated extra-radical mycelium (cord formers). Member of this group also correlated with high activities of classII peroxidases, known to facilitate break down of complex organic matter. Further evidence that these fungi act as “mycorrhizal white rotters” were obtained by amplification of peroxidase mRNA from soil extracts. The mRNA could be connected to mycorrhizal species by sequence homology. We propose that conditions of low nutrient availability favour the establishment of mycorrhizal species that are adapted to minimize immobilisation of nitrogen in stable organic pools. As their own mycelium represents a major sink for soil-derived nutrients, these species have to re-cycle their own biomass rapidly, in order to enable efficient delivery of nutrients to their host plants. They also possess potent oxidative enzymes that may be used to increase mobility of organic nutrients. As a side-effect of their highly efficient nutrient recycling, presence of these fungi also minimizes long term carbon sequestration in soils.

Population Genomics of Saccharomyces Yeasts: Ecology and Adaptation. Edward J. Louis. Ctr Genetics and Genomics, Univ Nottingham, Nottingham, United Kingdom.

The budding yeast, *Saccharomyces cerevisiae*, along with its close relatives, have only recently become reasonable models for the study of population genetics and evolution. This has been due to the lack of understanding of their natural history and ecology. Now that some understanding of budding yeast in nature is in hand, we can apply the powerful genetic and molecular tools available to questions of evolution through adaptation to ecological niches. The combination of population genomics and quantitative trait analysis has led to some understanding of the genetic architecture underlying traits which may be relevant to adaptation to particular environments in different yeast populations/species.

PLENARY SESSION ABSTRACTS

The mycorrhizal symbiosis as a network linking plants. Marc-André Selosse. Centre d'Ecologie Fonctionelle et Evolutive, Montpellier, France.

Most plants form mycorrhizal symbioses with soil fungi, which turn out to form networks between plants. Indeed, fungal individuals are large enough to colonize several root systems, and most mycorrhizal fungi are host-generalists that can link different plant species. The most dramatic evidence for such networks is the repeated emergence of mycoheterotrophy (MH) in plant evolution. MH plants are achlorophyllous and receive carbon (C) from surrounding green plants by way of shared mycorrhizal fungi. They recently made strong achievements due to two tools: molecular barcoding identified the fungi, and natural isotopic abundances (^{13}C, ^{15}N) confirmed which fungal mycorrhizal guild provides C. Temperate MHs belong to orchids and Monotropoideae (an Ericaceae subfamily) associate often with high specificity to basidiomycetes that form themselves ectomycorrhizae with surrounding trees. Intermediate evolutionary steps exist, in green orchids and Montropoideae that use C from mycorrhizal fungi in addition to their photosynthesis. This so-called mixotrophic nutrition depends on ectomycorrhizal fungi, and thus also on mycorrhizal networks. Phylogenies support that mixotrophy predisposed to evolution of MHs. In some green mixotrophic orchids, the survival of rare achlorophyllous variants further supports MH abilities, but the low fitness of these variants suggests that mixotrophy can be evolutionarily metastable. By contrast, tropical MH plants belong to diverse families, display lower specificities, and often associate to arbuscular mycorrhizal (AM) fungi. The isotopic fractionation for ^{15}N and ^{13}C along the [green plant-AM fungi-MH plants] continuum shows differences as compared to the same continuum for temperate MHs, which associate with ectomycorrhizal basidiomycetes. This supports different exchange mechanisms. Moreover, MHs associated to basidiomycetes have higher total N and ^{15}N content than autotrophic plants, while AM-associated MHs do not. I hypothesize that AM-associated MHs evolved mainly to support C nutrition, under selection of shaded, N-rich tropical forests. Conversely, basidiomycetes-associated MHs may have been first selected for N acquisition in N-limited, but less shaded, temperate forests. Thus, the convergent exploitation of mycorrhizal networks may result from different evolutionary pathways that depend on the biome.

Unraveling speciation and specialization processes in plant pathogenic fungi using comparative population genomics. Eva H Stukenbrock[1], Freddy B Christiansen[2], Julien Y Dutheil[1], Bruce A McDonald[3], Thomas Bataillon[2], Mikkel H. Schierup[2]. 1) Fungal Biodiversity, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Bioinformatics Research Center, Aarhus Univeristy, C.F. Moellers Alle 1110, 8000 Aarhus, Denmark; 3) Plant Pathology, ETH Zurich, LFW B16, 8092 Zurich, Switzerland.

The emergence of new fungal pathogens in managed ecosystems is an urgent matter of consideration. Studies have documented that natural ecosystems serve as reservoirs and sources of new crop infecting pathogens. We know, however, very little about the ecology and diversity of fungal pathogens in natural ecosystems. A goal of our research is to infer diversification and speciation processes of plant pathogens in natural and managed ecosystems. We study a species complex of plant pathogenic fungi including the wheat pathogen *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*). Speciation of *Z. tritici* was associated with wheat domestication and dates back to 10-12000 ya. Several closely related species of *Z. tritici* exist in natural grasslands in Iran. These wild grass pathogens co-exist and have over-lapping host ranges. In spite of their close relatedness, ecological or spatial factors have allowed speciation to occur. We have taken a comparative population genomics approach to study the underlying evolutionary processes that drive *Zymoseptoria* diversification. Our study includes full genome sequences from 52 fungal isolates representing four *Zymoseptoria* species. We perform population genomics analyses and document recent speciation times in the *Zymoseptoria* complex and present day small effective population sizes. Using within and between species rates of non-synonymous and synonymous variation we show a strong impact of natural selection in genome evolution of *Zymoseptoria spp*. This is at odds with the small effective population sizes estimated and suggests that population sizes were historically large but unstable. A significant finding is that speciation of *Z. tritici* did not entail an apparent loss of variation in spite of the homogenous agro-ecosystem where it has evolved. In contrast, we observe a dramatic loss of variation in the closest wild relative, *Z. pseudotritici*. The mosaic genome patterns in *Z. pseudotritici* are consistent with a very recent hybrid speciation event resulting from a cross between two divergent haploid individuals. We estimate that the hybridization occurred ~500 sexual generations ago between closely related, but isolated species. Based on the comparative population genomic analyses we reveal rapid evolution and distinct patterns of species evolution in natural and managed ecosystems.

Wednesday, March 13 3:00 PM–6:00 PM
Merrill Hall

Cell Signaling Involved in Fungal Development and Pathogenesis

Co-chairs: Naweed Naqvi and Stefanie Pöggeler

Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor. Alexander V. Michkov, Katherine A. Borkovich. Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

Heterotrimeric G proteins consist of alpha, beta and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) by the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. GDP/GTP exchange is facilitated by both cell surface G protein coupled receptors and cytosolic guanine nucleotide exchange factors (GEFs), such as RIC8. *Neurospora crassa* has three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1), and one G gamma (GNG-1). Interestingly, mutants lacking *gnb-1* or the cytosolic GEF *ric8* exhibit some defects in common with the *gna-1* deletion mutant, which may be explained by the reduced GNA-1 protein levels observed in these mutants. Previous studies in our laboratory showed that levels of *gna-1* mRNA are similar in wild type and mutants lacking *gnb-1* or *ric8*, consistent with a post-transcriptional mechanism. Using genetic and biochemical approaches, this study investigated the mechanism underlying regulation of GNA-1 stability in regards to GTP/GDP bound state and amount of protein (normal or overexpressed). The results demonstrate that levels of GNA-1 protein are not visibly reduced over 36 hours in a wild-type background after halting translation using cycloheximide, suggesting GNA-1 is very stable in wild type. To check stability of GDP or GTP bound GNA-1 in different backgrounds, we transformed mutants lacking the *gna-1* gene and *gnb-1* or *ric8* with a wild type (*gna-1*^{WT}) or constitutively active, GTPase-deficient *gna-1* allele (*gna-1*^{Q204L}). Overexpressing *gna-1*^{WT} (GDP bound) in a wild-type background increased the level of GNA-1 protein ~ 3 fold, while overexpression in a *gnb-1* mutant gave a nominal increase (~ 1.6x). Overexpressing *gna-1*^{Q204L} (GTP bound) in the *Dgnb-1* or *Dric8* backgrounds led to ~ 2 fold higher levels of GNA-1 compared to wild type. In summary, GNA-1 is very stable in wild type, but stability decreases dramatically in *gnb-1* and *ric8* deletion mutants. The GTP-bound G alpha protein is more stable in a *gnb-1* mutant background than GDP-bound GNA-1 protein.

The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in *Aspergillus*. Nak-Jung Kwon[1], Hee Soo Park[2], Seunho Jung[3], Sun Chang Kim[4], Jae-Hyuk Yu[1,2]. 1) Dept Bacteriology, University of Wisconsin, Madison, WI. USA; 2) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, USA,; 3) Department of Bioscience and Biotechnology, and Center for Biotechnology Research in UBITA, Konkuk University, Seoul, Republic of Korea; 4) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Dae-Jon, Republic of Korea.

Heterotrimeric G proteins (G proteins) govern growth, development, and secondary metabolism in various fungi. Here, we characterized *ricA*, which encodes a putative GDP/GTP exchange factor for G proteins in the model fungus *Aspergillus nidulans* and the opportunistic human pathogen *Aspergillus fumigatus*. In both species, *ricA* mRNA accumulates during vegetative growth and early developmental phases, but it is not present in spores. The deletion of *ricA* results in severely impaired colony growth and the total (for *A. nidulans*) or near (for *A. fumigatus*) absence of asexual sporulation (conidiation). The overexpression (OE) of the *A. fumigatus ricA* gene (*AfricA*) restores growth and conidiation in the D*AnricA* mutant to some extent, indicating partial conservation of RicA function in *Aspergillus*. A series of double mutant analyses revealed that the removal of RgsA (an RGS protein of the GanB Ga subunit), but not *sfgA*, *flbA*, *rgsB*, or *rgsC*, restored vegetative growth and conidiation in *AnricA*. Furthermore, we found that RicA can physically interact with GanB in yeast and *in vitro*. Moreover, the presence of two copies or OE of *pkaA* suppresses the profound defects caused by D*AnricA*, indicating that RicA-mediated growth and developmental signaling is primarily through GanB and PkaA in *A. nidulans.* Despite the lack of conidiation, *brlA* and *vosA* mRNAs accumulated to normal levels in the *ricA* mutant. In addition, mutants overexpressing *fluG* or *brlA* (OE*fluG* or OE*brlA*) failed to restore development in the *AnricA* mutant. These findings suggest that the commencement of asexual development requires unknown RicA-mediated signaling input in *A. nidulans*.

The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. Oezguer Bayram[1*], Oezlem Sarikaya Bayram[1], Yasar Luqman Ahmed[2], Jun-Ichi Maruyama[1,4], Oliver Valerius[1], Silvio Rizzoli[3], Ralf Ficner[2], Stefan Irniger[1], Gerhard Braus[1]. 1) Institute of Microbiology & Genetics, Department of Molecular Microbiology and Genetics, Georg-August-Universität, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Molecular Structural Biology, Institute for Microbiology and Genetics, Georg-August-Universität, Goettingen; 3) European Neuroscience Institute, Deutsche Forschungsgemeinschaft Center for Molecular Physiology of the Brain/Excellence Cluster 171, 37077 Göttingen; 4) Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from the plasma membrane to the nucleus. We show here that the module of the filamentous fungus Aspergillus nidulans (An) consists of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11, and the AnSte50 adaptor. The fungal MAPK module controls the coordination of fungal development and secondary metabolite production. It lacks the membrane docking yeast Ste5 scaffold homolog but similar to yeast the entire MAPK module interacts with each other at the plasma membrane. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 interacts with the conserved nuclear transcription factor AnSte12 to initiate sexual development and phosphorylates VeA which is a major regulatory protein required for sexual development and coordinated secondary metabolite production. Our data suggest that not only Fus3 but even the entire MAPK module complex of four physically interacting proteins can migrate from plasma membrane to nuclear envelope.

The developmental PRO40/SOFT protein participates in signaling via the MIK1/MEK1/MAK1 module in *Sordaria macrospora*. Ines Teichert[1], Eva Steffens[1], Nicole Schnab[1], Benjamin Fränzel[2], Christoph Krisp[2], Dirk A. Wolters[2], Ulrich Kück[1]. 1) General & Molecular Botany, Ruhr University Bochum, Bochum, Germany; 2) Analytical Chemistry, Ruhr University Bochum, Bochum, Germany.

Filamentous fungi are able to differentiate multicellular structures like conidiophores and fruiting bodies. Using the homothallic ascomycete *Sordaria macrospora* as a model system, we have identified a number of developmental proteins essential for perithecium formation. One is PRO40 [1], the homolog of *Neurospora crassa* SOFT, and this protein was employed for protein-protein interaction studies to gain insights into its molecular function. Data from yeast two hybrid experiments with PRO40 as bait show an interaction of PRO40 with the MAP kinase kinase (MAPKK) MEK1. MEK1 is a member of the cell wall integrity (CWI) pathway, one of three MAP kinase modules present in *S. macrospora*. The *S. macrospora* CWI pathway consists of MAP kinase kinase kinase (MAPKKK) MIK1, MAPKK MEK1 and MAP kinase (MAPK) MAK1, with additional upstream components, protein kinase C (PKC1) and RHO GTPase RHO1. Data from tandem affinity purification - MS experiments with PRO40 and MEK1 as bait indicate that PRO40 forms a complex with components of the CWI pathway. Analysis of single and double knockout mutants shows that PRO40, MIK1, MEK1 and MAK1 are involved in the transition from protoperithecia to perithecia, hyphal fusion, vegetative growth, and cell wall stress response. Differential phosphorylation of MAPKs in a pro40 knockout strain was detected by Western analysis. We propose that PRO40 modulates signaling through the CWI module in a development-dependent manner. Further interaction studies and complementation analyses with PRO40 derivatives provide mechanistic insight into the function of PRO40 domains during fungal development. [1] Engh et al. (2007) Eukaryot Cell 6:831-843.

A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrinal Signaling. Linqi Wang, Xunyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Microbes live mostly in a social community rather than in a planktonic state. Such communities have complex spatiotemporal patterns that require intercellular communication to coordinate gene expression. Here, we demonstrate that *Cryptococcus neoformans*, a model eukaryotic pathogen, responds to an extracellular signal in constructing its colony morphology. The signal that directs this community behavior is not a molecule of low molecular weight like pheromones or quorum sensing molecules, but a secreted protein. We successfully identified this protein as the conserved adhesin Cfl1 in the extracellular matrix. The released Cfl1 acts as an auto-induction signal to stimulate neighboring cells to phenocopy Cfl1-expressing cells. We propose that such adhesin/matrix-initiated communication system exists in divergent microbes and our work represents the first adhesin/matrix-mediated signaling mechanism in simple eukaryotes.

Surface recognition and appressorium morphogenesis in Magnaporthe oryzae. JinRong Xu. Dept Botany & Plant Pathology, Purdue Univ, West Lafayette, IN 47906.

Appressorium formation and penetration play critical roles in plant infection in the rice blast fungus and other foliar pathogens. In Magnaporthe oryzae, the cAMP signaling and Pmk1 MAP kinase pathways are known to regulate surface recognition, appressorium formation, penetration, and invasive growth. Like other filamentous ascomycetes, M. oryzae contains two genes encoding catalytic subunits of PKA. Whereas the cpkA mutant was delayed in appressorium formation and reduced in virulence, the cpk2 mutant had no detectable phenotypes except a slight reduction in conidiation. However, the cpkA cpk2 double mutant recently identified in our lab had distinct defects in growth, conidiation, appressorium formation, and plant infection. Detailed characterization of its phenotype is under the way and will be helpful to better understand the relationship between the cAMP signaling and Pmk1 pathways. For upstream signals, MSB2 functions as a surface sensor upstream from the Pmk1 pathway for regulating appressorium formation and penetration. In addition to its mucin and transmembrane domains, the cleavage domain and C-terminal cytoplasmic tail are important for Msb2 functions. Results from experiments aiming to determine cleavage and intracellular signaling of Msb2 will be presented. We also have used the msb2 or msb2 sho1 mutant to generate the double or triple mutants with CBP1 and PTH11, two other putative surface sensor genes. Phenotype characterization of these mutants will be used to determine the functional relationship of MSB2 with other surface sensors invovled in appressorium morphogenesis.

Plant cues promote stealth infection in fungal plant pathogens. Marie Nishimura. Plant-Microb Interact Unit, Natl Inst Agrobiol Sciences, Ibaraki, Japan.

Fungal cell wall, mainly composed of polysaccharides, is a major source of microbe-associated molecular patterns (MAMPs) which are recognized by host innate immune receptors. Although recognition of the fungal cell wall MAMPs, such as chitin oligomers, activates defense responses in plants, fungal plant pathogens invade the hosts likely by evading the host innate immunity. We have found that the ascomycete rice pathogen *Magnaporthe oryzae* accumulates a-1,3-glucan on the cell wall during infection. The accumulation of a-1,3-glucan was dependent on the cell wall integrity MAP kinase (Mps1) pathway, which was activated by a plant wax component. a-1,3-glucan was not essential for formation of infectious structures but was required for the successful infection by protecting the cells from plants' antifungal enzymes and by delaying the host defense responses. Furthermore, histocytochemical observation have revealed that the ascomycete *Cochlioborus miyabeanus* and the basidiomycete *Rhizoctonia solani* have also accumulate a-1,3-glucan on the cell walls specifically during plant invasion. Thus, plant cues appear to trigger surface accumulation of a-1,3-glucan in these fungi. In addition, rice plants secreting bacterial a-1,3-glucanase rapidly induced defense responses against these pathogens and showed multiple fungal disease resistance. Considering that a-1,3-glucan is non-degradable in many plants, our study suggested that masking cell wall surfaces with a-1,3-glucan is a stealth infection strategy commonly used by fungal plant pathogens. Our study also indicated that recognition of plant cues play an important role in promoting stealth infection in fungal plant pathogens.

Unravelling the GTPase polarity complex in *Claviceps purpurea*. Andrea Herrmann[1], Janine Schürmann[1], Britta Tillmann[2], Michael Bölker[2], Paul Tudzynski[1]. 1) IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany; 2) Philipps-Universität, Karl-von-Frisch-Strasse 8, 35032 Marburg, Germany.

Claviceps purpurea is a plant pathogen infamous for its production of toxic alkaloids on infected host plants like barley. Consumption of infected grains leads to severe symptoms up to the death of the patient. Infection patterns are complex and the topic of intensive research. One interesting aspect is the strict polarity of the hyphal growth during the first infection stage which seems to be crucial for the non-recognition of *C. purpurea* as a pathogen by the host. To address the question of the importance of polarity the structure and dynamics of the polarity complex are the focus of this work. The guanine nucleotide exchange factors (GEFs) Cdc24 and Dock180 belong to different families, Cdc24 being a member of the Dbl GEF family and Dock180 a CZH GEF. Cdc24-GFP localises cytosolically and to hyphal tips whereas Dock180-GFP is present in small vesicles in the hypha, though concentrated at the tip region, too. Cdc24 DHPH domains are able to activate the small GTPases Rac and Cdc42 of *C. purpurea* and *U. maydis In vitro*, whereas the catalytic domain of Dock180 only activates Rac in both organisms. Despite the proven activation Cdc24 does not interact with any GTPase in yeast two hybrid assays. Dock180 shows a weak interaction with Rac and the two p21-activated kinases (PAKs) Ste20 and Cla4. Thus, both GEFs do not share many characteristics apart from their GEF activity. The PAKs Ste20 and Cla4 and the scaffold protein Bem1 are involved in the polarity complex, too. Ste20 localises to hyphal tips and interacts with Cdc42 in a loading status dependent manner, whereas Cla4 is the main partner of Rac. Other interactions of Ste20 with Dock180 and Cla4 could also be shown. Bem1 is present in the cytosol - concentrated at the hyphal tip - and links most of the proteins of the polarity complex as interactions with Cdc24, Cla4, Ste20 and Dock180 have been detected. Taken together we postulate at least two different polarity complexes, the Rac complex and the Cdc42 complex. Both are gathered by Bem1, but Cla4 is the main partner of Rac, whereas Ste20 plays a similar role for Cdc42. Dock180 is mainly linked to Rac, Cdc24 can be active in both complexes. We are interested in the spatial and temporal formation and regulation of these complexes and its influence on polarity and virulence which will be the subject of further studies.

Wednesday, March 13 3:00 PM–6:00 PM
Chapel

Genetics and Genomics of Interactions with Bacteria, Insects and Plants

Co-chairs: Nemat Keyhani and Christian Hertweck

Endophytic insect parasitic fungi feed insect-derived nitrogen to plants. M.J. Bidochka, S.W. Behie. Brock University, St. Catharines, ON, Canada.

Metarhizium is a fungus with a bifunctional lifestyle: it is a common plant endophyte and is also a pathogen to a large number of insects, which are a source of nitrogen. It is possible that the endophytic capability and insect pathogenicity of Metarhizium are coupled to provide an active method of nitrogen transfer to plant hosts via fungal mycelia. We used soil microcosms to show the ability of M. robertsii to translocate insect-derived nitrogen to plants. Insects were injected with 15N-labeled nitrogen, and we tracked the incorporation of 15N into amino acids in two plant species. We also investigated the exchange of plant carbon for insect-derived nitrogen in this symbiosis. Unlike mycorrhizal fungi, Metarhizium, is not a fastidious fungus and is easily genetically manipulated. We also performed gene knockout experiments on carbon and nitrogen transporters to test if the exchange of carbon and nitrogen is reciprocal in this fungus-plant symbiosis.

Genotype-Environment Interactions and the Interplay Between Climate Change and Plant-Fungal Symbioses. Rusty J. Rodriguez[1,2,3], Yong Ok Kim[3], Claire Woodward[3], Leesa Wright[2], Regna Redman[2,3]. 1) Symbiogenics, Seattle, WA; 2) Adaptive Symbiotic Technologies, Seattle, WA; 3) University of Washington, Biology, Seattle, WA.

Symbiotic associations span a continuum from parasitism to mutualism and the outcome of specific associations is context driven based on inter-genomic interactions and environmental factors. These factors will determine the ability plants and animals to adapt to a changing climate. For example, plants in natural ecosystems adapt to abiotic stress by forming symbiotic associations with fungal endophytes that confer stress tolerance. Without the endophytes, the plants are not stress tolerant and do not survive in the habitats to which they are adapted. Symbiotically conferred stress tolerance typically occurs in a habitat-specific manner, a phenomenon we designate Habitat Adapted Symbiosis (HAS). Although several biochemical processes have been correlated to plant stress tolerance, few processes correlate with symbiotically conferred stress tolerance. Symbiotically conferred stress tolerance involves altered plant gene regulation, increased metabolic efficiency, and an increased ability to manage reactive oxygen species. I will describe how fungal endophytes adapt plants across environmental gradients and present a working model for symbiotically conferred stress tolerance.

Chemical mediators of pathogenic and mutualistic bacterial-fungal interactions. Kirstin Scherlach. Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, HKI Beutenbergstr. 11a 07745 Jena, Germany.

Natural products play a key role in symbiotic interactions between microorganisms and higher organisms. Their function may range from signaling compounds in mutualism to virulence factors and toxins in pathogenic relationships. In many cases the chemical basis of such interactions is still unknown. By combining genome mining, bioinformatic analyses and chemical analytical techniques we uncovered the biosynthesis of a number of virulence factors and toxins from bacterial-fungal associations that impact agriculture, medicine and biotechnology. We elucidated an unprecedented case of symbiotic cooperation where a fungus tailors a pathogenicity factor provided by its endosymbiotic bacteria to increase its phytotoxic potency. This toxin (rhizoxin) causes rice seedling blight, a plant disease accounting for severe losses in agriculture. Another bacterium, *Janthinobacterium agaricidamnosum*, initiates soft rot of cultured mushrooms. With the help of imaging mass spectrometry we discovered a peptide toxin that not only contributes to pathogenicity but also displays potent antifungal activity against major human pathogens. Furthermore, we identified the molecular basis for the biosynthesis of a highly toxic polyketide produced by *Burkholderia gladioli*, a common contaminant of the food fermentation fungus *Rhizopus oligosporus*. Bongkrekic acid is a respiratory toxin that efficiently inhibits adenine nucleotide translocase. Through sequencing of the bacterial genome and functional analyses of the biosynthetic genes new insights into the process of polyketide assembly were gained. The discovery of these secondary metabolites as mediators of bacterial-fungal interaction and their biogenetic origins not only facilitates the understanding of complex ecological processes but also opens avenues to the development of new drug candidates and potential bio-control agents against crop diseases.

Comparative genomic analysis of entomopathogenic fungi. Guohua Xiao, Qiang Gao, Peng Zheng, Xiao Hu, Chengshu Wang. Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China.

There are about one thousand of fungal species capable of infecting and killing insects, spiders and mites. The species like *Metarhizium anisopliae*, *M. robertsii*, *M. acridum* and *Beauveria bassiana* has been developed as environmentally friendly biological control agents against different insect pests. The caterpillar pupae specific fungus *Cordyceps militaris*, however, has been used a traditional Chinese Medicine for hundreds of years. We sequenced the genome of model entomopathogenic fungi *Metarhizium* spp., *B. bassiana* and *C. militaris* and conducted comparative genomic studies. We found that the insect pathogens have a strikingly larger proportion of genes encoding secreted proteins, particularly proteases, than other sequenced fungi. A phylogenomic analysis confirmed that fungal entomopathogenicity has evolved multiple times so similar expansion of families of proteases and chitinases reflects a convergent evolution. Like that of *C. militaris* which can readily performs a sexual life, a single mating type locus identified in clonally reproductive species *Metarhizium* and *Beauveria* indicated that the later are sexually heterothallic. High throughput transcriptome analysis indicated that the pathogens could regulate transcriptional responses with fine-tuned gene sets for host recognition, development and adaptation to different niches. The information from our studies advanced the understanding of the evolution of fungal entomopathogenicity and will benefit future molecular studies of fungus-host interactions and thereby facilitate the development of cost-effective mycoinsecticides.

Synergistic interactions between leaf-cutting ants and their fungal symbiont facilitate degradation of plant substrate. Morten Schiøtt[1], Henrik H. de Fine Licht[2], Adelina Rogowska-Wrzesinska[3], Pepijn Kooij[1], Peter Roepstorff[3], Jacobus J. Boomsma[1]. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Department of Mycology, Lund University, Lund, Sweden; 3) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.

About 50 million years ago a single ancestor of today's more than 230 species of fungus-growing ants committed herself irreversibly to farming fungi for food instead of being a hunter-gatherer as most other ants. However, the white-rot litter decomposing Leucocoprini (Agaricales) that were domesticated remained mostly uncommitted to the symbiosis until a single lineage became an obligate symbiont of a derived clade of these ants - the so called higher attines. Coevolution of ants and fungi has subsequently produced specific adaptations in both partners, including the development of special hyphal tips (gongylidia) of the fungus on which the ants feed. Recent work has shown that many fungal enzymes pass through the ant digestive system unharmed to be mixed (as fecal fluid) with the fresh leaf pulp that the ants deposit on top of their gardens. To understand the function of this form of fungal enzyme transfer, we have used state of the art proteomics and high-throughput genome sequencing to identify the proteins found in the ant fecal fluid. Fecal proteins of *Acromyrmex* leafcutter ants were separated with SDS-PAGE followed by tandem mass spectrometry, and the resulting peptide tags were used as queries to Blast-search a low coverage genome sequence of the fungal symbiont. Using this strategy we identified 34 protein sequences encoded by the fungal genome. Enzyme assays for selected fecal proteins showed that they functionally disappeared from the fecal droplets when the ants were deprived of their fungal symbiont. We further used qPCR to establish that many of these proteins are more highly expressed in gongylidia than in mycelium, suggesting that they have been actively selected to be ingested by the ants. A substantial fraction of the fecal proteins are enzymes that are widely used by plant-pathogens to break down cell walls to access the easily degradable nutrients inside living cells. Of special interest is the finding of a polyphenol-oxidizing laccase enzyme that shows signs of positive selection in the higher attine ant symbionts, and may be an important prerequisite for the ability to cope with the polyphenols present in plant tissues. The results indicate that the leafcutter ants and their fungal symbionts have evolved traits-syndromes that are partially convergent with those found in plant-pathogenic fungi.

Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains. Charissa de Bekker, David Hughes. Biology and Entomology, Center for Infectious Disease Dynamics,Pennsylvania State University, State College, PA.

Fungal entomopathogens rely on cellular heterogeneity during the different stages of insect host infection. Their pathogenicity is exhibited through the secretion of secondary metabolites. Infection strategies of this group of environmentally important fungi can thus be studied by analyzing their metabolome. Next to generalists such as *Beauveria bassiana* and *Metarhizium anisopliae*, specialist species exist that are able to control host behavior. One of the most dramatic examples is the death grip of ants infected by *Ophiocordyceps unilateralis*, where ants are being used as a vehicle and finally bite into vegetation before dying, aiding fungal spore dispersal after death. To establish this the fungus must not only overcome the immune system of the host, but also manipulate the brain and atrophy the muscles. To date, most work on manipulation of host behavior has described the ant's behavior, leaving the molecular processes from the fungal point of view unresolved. To start unraveling the mechanisms underlying this phenomenon we are combining metabolite profiling with an *ex vivo* insect tissue culturing system that allows us to study fungal metabolites secreted in different parts within the host. Using this technique we established that *B. bassiana* and *M. anisopliae*, and *O. unilateralis* heterogeneously react to brain and muscle tissue by secreting a significantly different array of metabolites. The combination of these approaches with a concrete understanding of the host-parasite interaction in nature is allowing us to understand both the diversity of secondary metabolites as well as make discoveries regarding the temporal dynamics these fungi employ when releasing metabolites that affect the host. *This project is financed by the Marie Curie International Outgoing Fellowships and Penn State University* .

Trichoderma rhizosphere's competency, endophytism and plant communication: A molecular approach. Artemio Mendoza[1], Johanna Steyaert[1], Natalia Guazzone[1], Maria Fernanda Nieto-Jacobo[1], Mark Braithwaite[1], Robert Lawry[1], Damian Bienkowski[1], Christopher Brown[2], Kirstin MacLean[1], Robert Hill[1], Alison Stewart[1]. 1) Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; 2) Biochemistry Department and Genetics Otago, University of Otago, New Zealand.

Establishment of root symbiosis is one the key drivers of biocontrol success for members of the fungal genus *Trichoderma*. This root symbiosis is described as a two-step process, whereby *Trichoderma* species colonise the soil surrounding the root (rhizosphere) and then penetrate the root tissue and establish an endophytic relationship. The ability to colonise and then proliferate over time within the rhizosphere is termed rhizosphere competence (RC). There have been numerous reports of *Trichoderma* biocontrol strains which persist within the rhizosphere for the growing season of the crop plant. Our results strongly suggest that RC is widespread among members of the genus *Trichoderma* and that RC interactions are strain and host plant specific. For endophytes and their host plants to maintain a mutualistic relationship requires a constant molecular dialogue between the organisms involved. For example, the fungal-derived phytohormone, indole acetic acid (IAA), plays an important role in signalling between *Trichoderma* and the model plant *Arabidopsis thaliana*. There are however, additional, currently unknown, chemical signals which may be even more important for a positive interaction between *Trichoderma* and plants. By using a soil-maize-*Trichoderma* as a model system in in situ sterile conditions we are currently analysing the RC and endophytism transcriptomes of two *Trichoderma* species: *T. virens* and *T. atroviride*. Using a combination of bioinformatics, quantitative RT-PCR (for stage specific genetic markers from *Trichoderma*) and fluoro-labelled *Trichoderma* strains we are currently identifying and analysing promising *Trichoderma* candidates involved in endophytism and RC. A comprehensive panorama of the *Trichoderma*-soil-plant interaction will be discussed in this conference.

Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance. Therese Wohlschlager[1], Stefanie Schmieder[1], Alex Butschi[2], Paola Grassi[3], Alexander Titz[4], Stuart Haslam[3], Michael Hengartner[2], Markus Aebi[1], Markus Künzler[1]. 1) Institute of Microbiology, ETH Zürich, Switzerland; 2) Institute of Molecular Life Sciences, University of Zürich, Switzerland; 3) Division of Molecular Biosciences, Imperial College, London, United Kingdom; 4) Department of Chemistry, University of Konstanz, Germany.

The defense of fungi against fungivores is largely based on the production of intracellular toxins. A significant proportion of these toxins are peptides and proteins that are synthesized by the ribosome and stored in the cytoplasm. Protein toxins include lectins that target specific glycoepitopes in the intestine of the fungivore upon ingestion and kill the fungivore by a yet unknown mechanism. In our laboratory, we focus on the functional characterization of fungal protein toxins that are directed against nematodes. We use the model nematode *Caenorhabditis elegans* to identify the targets and to study the toxicity mechanism of these fungal defense effector proteins in the nematode. In addition, we employ the fungivorous nematodes *Aphelenchus avenae* and *Bursaphelenchus willibaldi* to study the diversity, the functional significance and the transcriptional regulation of these proteins in the fungus. Recently, we identified a nematotoxic lectin from the mushroom *Laccaria bicolor* that is homologous to animal lectins involved in innate immunity against bacteria. We found that the nematotoxicity of the lectin is based on its specific binding to methylated fucose residues on nematode N-glycans. Among animals, this epitope is only present in worms and molluscs but not in insects or vertebrates. We performed affinity chromatography of *C. elegans* whole worm protein extracts using the *L. bicolor* lectin and other nematotoxic fungal lectins recognizing protein-bound glycans. The results of this analysis suggest that these lectins target the same set of glycoproteins in the nematode intestine and may confer toxicity by a common mechanism. In order to address the functional significance of these proteins for fungal defense against fungivorous nematodes, we expressed some of the fungal proteins displaying toxicity towards *C. elegans*, in the filamentous ascomycete *Ashbya gossypii*. These transformants were fed to *A. avenae* and the propagation of the fungivorous nematode on the various transformants was determined. Expression of some effector proteins significantly inhibited propagation of the nematode suggesting that these proteins have a role in fungal defense against these organisms. Experiments addressing the relative fitness of the various *A. gossypii* transformants upon selective pressure of feeding by *A. avenae* are under way.

Wednesday, March 13 3:00 PM–6:00 PM
Heather

Membrane Trafficking and Molecular Organization

Co-chairs: Vicky Sophianopoulou and Gero Steinberg

Distinct secretion systems operate during biotrophic invasion by the rice blast fungus, *Magnaporthe oryzae*. Barbara Valent[1], Martha Giraldo[1], Chang Hyun Khang[1,4], Yasin Dagdas[2], Yogesh Gupta[2], Thomas Mentlak[2,5], Mihwa Yi[1], Melinda Dalby[1], Hiromasa Saitoh[3], Ryohei Terauchi[3], Nicholas Talbot[2]. 1) Dept Plant Pathology, Kansas State Univ, Manhattan, KS; 2) School of Biosciences, Univ of Exeter, Exeter, U.K; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, Japan; 4) Current Address: Dept of Plant Biology, Univ of Georgia, Athens, GA; 5) Current Address: Cambridge Consultants Ltd, Cambridge, U.K.

During biotrophic invasion, *Magnaporthe oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the cytoplasm of the rice cells, and apoplastic effectors, which remain in the extracellular space between the fungal cell wall and the rice plasma membrane. BICs localize in front of the tips of filamentous hyphae that enter rice cells, and remain subapically beside the first bulbous invasive hyphal cells after hyphal differentiation. In contrast, secreted apoplastic effectors uniformly outline the entire bulbous invasive hypha. We have determined that cytoplasmic effector genes were highly up-regulated in the BIC-associated cells at early invasion stages, and that effector promoters played the major role in determining preferential BIC localization of cytoplasmic effectors. Subapical BIC-associated hyphal cells continued to express protein secretion machinery components while invasive hyphae grew elsewhere in the host cell, suggesting that these subapical invasive hyphal cells are involved in active secretion. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocked secretion of apoplastic effectors, but not secretion of cytoplasmic effectors. Pathogen mutants that failed to express exocyst complex components or a t-SNARE were defective in secretion of cytoplasmic effectors, as well as defective in pathogenicity. In contrast, secretion of apoplastic effectors was not impaired in these mutants. Our data suggest that *M. oryzae* possesses distinct secretory mechanisms for targeting cytoplasmic and apoplastic effectors during rice invasion.

The cellular role of early endosome motility in *Ustilago maydis*. Yujiro Higuchi[1], Peter Ashwin[2], Gero Steinberg[1]. 1) Biosciences, University of Exeter, Exeter EX4 4QD, UK; 2) Mathematics Research Institute, University of Exeter, Exeter EX4 4QF, UK.

Early endosomes (EEs) are dynamic organelles that move along microtubules, which is mediated by the motor proteins kinesin-3 and dynein. Despite our growing knowledge about the mechanistics of motion, the physiological significance of EE motility remains elusive. A recent study suggested that RNA-binding proteins travel on EEs, which might support local protein translation at the hyphal tip. However, evidence for apical translation is missing. Here, we investigate the distribution of ribosomes, using native levels of ribosomal proteins. We will summarize our findings on protein translation and will discuss the role of EE-dependent transport of RNA-binding proteins in the light of our findings.

The arrestin-like protein ArtA is essential for ubiquitylation and endocytosis of the UapA transporter in response to both broad-range and specific signals. George Diallinas, Mayia Karachaliou, Sotiris Amillis, Minos Evangelinos, Alexandros Kokotos. Faculty of Biology, University of Athens, Athens, Greece.

We investigated the role of all arrestin-like proteins of Aspergillus nidulans in respect to growth, morphology, sensitivity to drugs and specifically for the endocytosis and turnover of the uric acid-xanthine transporter UapA. All arrestin null mutants are viable showing wild-type growth and morphology, except one which is affected in conidiospore production, but several have modified profiles in respect to N or C source utilization and drug sensitivity. A single arrestin, ArtA, is essential for HulARsp5-dependent ubiquitination and endocytosis of UapA in response to ammonium or substrates. Genetic analysis further showed that residues 545-561 of the UapA C-tail, which includes a critical di-acidic motif, is required for efficient UapA endocytosis. Mutational analysis of ArtA shows that the N-terminal region (2-123) and both PY elements are essential for its function. ArtA undergoes HulA-dependent ubiquitination at residue Lys343 and this modification is critical for the efficiency of UapA ubiquitination and endocytosis, especially in response to ammonium. Lastly, we show that ArtA is essential for vacuolar turnover of transporters specific for purines (AzgA) or L-proline (PrnB), but not for an aspartate/glutamate transporter (AgtA). Our results are discussed within the frame of recently proposed mechanisms on how arrestins are activated and recruited for ubiquitination of transporters in response to broad range signals, but also put the basis for understanding how arrestins, such as ArtA, regulate the turnover of a specific transporter in the presence of its substrates.

Escaping the hustle - zones of differential protein turnover in the yeast plasma membrane. Guido Grossmann[1,2], Vendula Stradalova[3], Michaela Blazikova[3], Miroslava Opekarová[4], Jan Malinsky[3], Widmar Tanner[5]. 1) Center for Organismal Studies, University of Heidelberg, Heidelberg, Germany; 2) Department for Plant Biology, Carnegie Institution for Science, Stanford, CA; 3) Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic; 4) Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; 5) Cell biology and plant biochemistry, University of Regensburg, Regensburg, Germany.

The plasma membrane (PM) consists of specialized domains that differ in their protein distribution, lipid composition and structure, and that are essential for PM functions such as membrane transport or signal perception and transduction. The mechanisms that establish and maintain this heterogeneity are still largely unknown but involve interactions between membrane constituents, local modification of the membrane structure, tethering to other cellular components like ECM/cell wall or cytoskeleton, and polarized exo- and endocytosis. In the PM of bakers yeast, stable rod-shaped membrane invaginations exist, called membrane compartment of Can1 (MCC), that exhibit a specific composition of lipids and proteins, and are stabilized by a protein structure called eisosome. Chemical and genetic screens, revealed important roles of the membrane potential, lipid composition, and protein scaffolds in organizing the PM into specialized domains. The distribution of MCC domains further determines the distribution of the PM-associated cortical ER that dynamically covers large areas of the PM. Mapping of endocytic events revealed very low rates of clathrin-mediated endocytosis in PM areas covered by cER and within MCC. The formation of such "quiet zones" provides a mechanism for membrane domain formation through local confinement of membrane turnover.

Whole-genome sequencing identifies novel alleles of genes required for organelle distribution and motility in *Aspergillus nidulans*. Kaeling Tan, Anthony Roberts, Martin Egan, Mark Chonofsky, Samara Reck-Peterson. Cell Biology, Harvard Med Sch, Boston, MA.

Many organelles are transported long distances along microtubules in eukaryotic organisms by dynein and kinesin motors. To identify novel alleles and genes required for microtubule-based transport, we performed a genetic screen in the filamentous fungus, *Aspergillus nidulans*. We fluorescently-labeled three different organelle populations known to be cargo of dynein and kinesin in *Aspergillus*: nuclei, endosomes, and peroxisomes. We then used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Using whole-genome sequencing, we found a number of single nucleotide polymorphisms (SNPs) that resulted in misdistribution of peroxisomes, endosomes, or nuclei. Some of these SNPs were novel alleles of cytoplasmic dynein/ *nudA*, Arp1/ *nudK* (dynactin), Lis1/ *nudF*, and kinesin-1/ *kinA*. Here, we characterize the *in vivo* transport defects in these novel mutants and analyze the single molecule *in vitro* motility properties of purified mutant motor proteins. We also describe our methods for using whole genome sequencing as a tool in mutagenesis studies in *A. nidulans*.

Dynamics of exocytic markers and cell wall alterations in an endocytosis mutant of *Neurospora crassa*. Rosa R. Mouriño-Pérez, Ramón O. Echauri-Espinosa, Arianne Ramírez-del Villar, Salomón Bartnicki-García. Microbiology Department, CICESE, Ensenada, B.C., Mexico.

Morphogenesis in filamentous fungi depends principally on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. Previously, we found that coronin deletion (*Neurospora crassa* mutant, D*crn-1*) causes a decrease in endocytosis (measured by the rate of uptake of FM4-64) together with marked alterations in normal hyphal growth and morphogenesis accompanied by irregularities in cell wall thickness. The absence of coronin destabilizes the cytoskeleton and leads to interspersed periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. By confocal microscopy, we found that CRIB-GFP was present In wild-type hyphae as a thin hemispherical cap under the apical dome, i. e. when growing in a polarized fashion and with regular hyphoid morphology. In the D*crn-1* mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the D*crn-1* mutant correlated with the morphological irregularity of the hyphae. We found that the D*crn-1* mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the D*crn-1* mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

"The vacuole" of Neurospora crassa may be composed of multiple compartments with different structures and functions. Barry J. Bowman[1], Emma Jean Bowman[1], Robert Schnittker[2], Michael Plamann[2]. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Department of Biology, University of Missouri, Kansas City, KA.

The structure of the "vacuole" in *Neurospora crassa* and other filamentous fungi is highly variable with cell type and position in the hypha. Large spherical vacuoles are typically observed in older hyphal compartments, but approximately 100 microns behind the hyphal tip, vacuolar markers are seen in a dynamic network of thin tubules. At the edge of this network nearest the tip, a few distinct round organelles of relatively uniform size (2-3 microns) have been observed (Bowman *et al.* Eukaryotic Cell 10:654). The function of these round organelles is unknown, although the vacuolar ATPase and a vacuolar calcium transporter are strongly localized there. To help identify organelles we have tagged SNARE proteins and Rab GTPases with GFP and RFP. Several of these tagged proteins (sec-22, rab-7, rab-8) appear in the tubular vacuolar network and in the membrane of the round organelles. A unique aspect of the round organelles is their association with dynein and dynactin (Sivagurunathan *et al.* Cytoskeleton, 69:613). In strains with mutations in the tail domain of the dynein heavy chain the dynein is often seen in clumps. This aggregated dynein appears to be tightly associated with (and possibly inside) the round organelles, but not in the tubular vacuolar network. Further analysis of the location of SNARE and Rab proteins may help to identify the function of the round organelles.

Wednesday, March 13 3:00 PM–6:00 PM
Fred Farr Forum

Genome Defense, Epigenetics and RNAi

Co-chairs: Patrick Shiu and Sven Saupe

Meiotic silencing by unpaired DNA in *Neurospora*. Thomas M. Hammond[1], Hua Xiao[2], Erin C. Boone[2], Logan M. Decker[2], David G. Rehard[2], Seung A. Lee[2], Tony D. Perdue[3], Patricia J. Pukkila[3], Patrick K. T. Shiu[2]. 1) School of Biological Sciences, Illinois State University, Normal, IL; 2) Division of Biological Sciences, University of Missouri, Columbia, MO; 3) Department of Biology, University of North Carolina, Chapel Hill, NC.

Neurospora crassa has a cytoplasm that is shared by the entire hyphal network, making it particularly vulnerable to attack by repetitive elements. Accordingly, several surveillance mechanisms are in place to protect the genome integrity of the fungus. For example, if a gene is lacking a partner during homolog pairing in meiosis, all copies of this gene are silenced by a process known as meiotic silencing by unpaired DNA (MSUD). MSUD requires common RNAi proteins (e.g., RNA-directed RNA polymerase, Dicer, and Argonaute) and may work as follows: an unpaired gene triggers the production of an aberrant RNA molecule, which is made double-stranded (by SAD-1) and processed into siRNAs (by DCL-1). These siRNAs are subsequently used to destroy complementary mRNAs (by SMS-2). The aforementioned proteins colocalize in the perinuclear region, possibly forming a silencing complex that inspects and processes RNA molecules as they exit the nucleus. We will discuss the recent advances in our understanding of this unique silencing pathway.

Mechanism of quelling, a small RNA-mediated gene silencing pathway. Zhenyu Zhang, Shwu-Shin Chang, Yi Liu. Dept Physiology, Univ Texas SW Med Ctr, Dallas, TX.

RNAi is a conserved gene silencing mechanism from fungi to mammals. Quelling is an RNAi-related phenomenon that post-transcriptionally silences repetitive DNA and transposon in Neurospora. We previously identified a type of DNA damage-induced small RNA called qiRNAs that originate from ribosomal DNA. To understand how small RNAs are generated from repetitive DNA, we carried out a genetic screen to identify genes required for qiRNA biogenesis. Factors directly involved in homologous recombination (HR) and chromatin remodeling factors required for HR are essential for qiRNA production. HR is also required for quelling, and quelling is also the result of DNA damage, indicating that quelling and qiRNA production share a common mechanism. Together, our results suggest that DNA damage triggered HR-based recombination allows the RNAi pathway to recognize repetitive DNA to produce small RNA.

SIS, a sex genome defense mechanism operating in *Cryptococcus neoformans*. Xuying Wang, Sabrina Darwiche, Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans is a human fungal pathogen that undergoes a dimorphic transition from yeast to hyphae during **a**-a opposite-sex mating and a-a unisexual reproduction (same-sex mating). Infectious spores are generated during both processes. We previously identified a sex induced silencing (SIS) pathway in the *C. neoformans* serotype A var. *grubii* lineage, in which tandem transgene arrays trigger RNAi-dependent gene silencing at a high frequency during **a**-a opposite-sex mating, but at an ~250-fold lower frequency during asexual mitotic vegetative growth. Here we report that SIS also operates during a-a unisexual reproduction. A self-fertile strain containing either *SXI2***a**-*URA5* or *NEO-URA5* transgene arrays exhibited an elevated silencing frequency during solo and unisexual mating compared with mitotic vegetative growth. We also found that SIS operates at a similar efficiency on transgene arrays of the same copy number during either a-a unisexual reproduction or **a**-a opposite-sex mating. *URA5*-derived small RNAs were detected in the silenced progeny of a-a unisexual reproduction and RNAi core components were required, providing evidence that SIS induced by same-sex mating is also mediated by RNAi via sequence-specific small RNAs. This study, together with our previous finding of SIS in **a**-a opposite-sex mating of the *C. neoformans* serotype A var. *grubii* lineage, demonstrates that SIS is a conserved process between the divergent *C. neoformans* serotype A and serotype D sibling species. In each case, our data show that the SIS RNAi pathway operates to defend the genome via squelching transposon activity during the sexual cycles. Thus, our discovery of SIS brings a fresh perspective to meiotic silencing involving the upregulation of RNAi pathways as a strategy to guard genomic integrity during sex. More importantly, the presence of SIS in both a-a unisexual reproduction and **a**-a opposite-sex mating indicate that SIS may be triggered by the shared pheromone sensing Cpk1 MAPK signal transduction cascade. Ongoing studies focus on defining at a mechanistic level how the SIS RNAi pathway is initiated, including identifying new components involved in SIS.

Fungi use prion folds for signal transduction processes involving STAND proteins. Asen Daskalov, Khalid Salamat, Sven J. Saupe. CNRS, IBGC UMR5095, BORDEAUX, AQUITAINE, France.

Prions are proteins embedding genetic information into their structural state. Generally, those proteins exist in a soluble state and sporadically as infectious amyloid aggregates. Podospora anserina's [Het-s] is one of the best characterized fungal prions with a remarkably high prevalence in wild populations. [Het-s] functions in vegetative incompatibility - a biological process occurring during anastomosis between two genetically incompatible strains. The HET-s protein exists in a soluble state - [Het-s*] - or can switch to an aggregated amyloid state - [Het-s] - the prion form. When an [Het-s] prion infected strain fuses with a strain expressing the alternative allelic variant of the het-s locus - het-S - a cell death reaction of the heterokaryon occurs. Recent studies shed light on the mechanism of [Het-s]/HET-S incompatibility reaction. Differing by 13 amino acids both proteins shares a two domain architecture; a globular N-terminal domain called HeLo and a C-terminal Prion Forming Domain (PFD). The latter is able to adopt a b-sheet rich conformation with a specific b-solenoid fold. It has been demonstrated that in presence of [Het-s] amyloid fibers HET-S turns into a pore-forming toxin: transconformation of the HET-S PFD by [Het-s] fibers triggers the refolding of the HET-S HeLo domain, inducing the cell death reaction. In an attempt to better characterize the conserved features of the [Het-s] b-solenoid fold and identify new distant homologues of HET-S/s, we have generated a minimal consensus sequence motif of it. Surprisingly, the second best hit in a BLASTp search is in the N-terminal region (3-23) of the product encoded by nwd2, the immediately adjacent gene to het-S. NWD2 is a STAND protein. STAND proteins form signal transducing hubs through oligomerization upon ligand recognition. That in mind and several bioinformatics observations led us to propose that HET-S and NWD2 are functional partners in various filamentous fungal species using the amyloid fold in a signal transducing pathway. We will present experimental evidence that NWD2 is able to trigger HET-S toxicity in

much the same way as [Het-s] does. Further in silico analysis identify a number of these STAND/prion-like gene pairs and suggest that signal transduction through an amyloidal prion-like fold is a general widespread mechanism in fungi.

Regulation of white and opaque cell-type formation in Candida albicans by H3K56 acetylation and nucleosome assembly factors CAF-1 and HIR. John S. Stevenson, Haoping Liu. Department of Biological Chemistry, University of California, Irvine, Irvine, CA.

CAF-1 and HIR are highly conserved histone chaperone protein complexes that function in the assembly of nucleosomes onto chromatin. CAF-1 is characterized as having replication-coupled nucleosome activity whereas the HIR complex can assemble nucleosomes independent of replication. Histone H3K56 acetylation, controlled by the acetyltransferase Rtt109 and deacetylase Hst3, also plays a significant role in nucleosome assembly. How different cell types with the same genotype are formed and heritability maintained is a fundamental question in biology. We utilized white-opaque switching in Candida albicans as a system to study mechanisms of cell-type formation and maintenance. Opaque cell specification is under the control of interlocking transcriptional feedback loops, with Wor1 being the master regulator. We showed that H3K56 acetylation plays an important role in the regulation of white-opaque switching. The rtt109D/D mutant is defective in stochastic and environmentally stimulated white-opaque switching and cannot maintain opaque cell type. Inhibition of Hst3 by nicotinamide induces opaque cell formation in Rtt109 dependent manner. The Hst3 level is down-regulated in the presence of genotoxins and ectopic expression of HST3 blocks genotoxin induced switching, providing a pathway for genotoxin induced white-opaque switching. We now show that CAF-1 and HIR modulate white-opaque switching frequencies in a H3K56 acetylation associated manner. Unique to C. albicans, the cac2D/D mutant shows increased sensitivity to the Hst3 inhibitor nicotinamide, while the rtt109D/D cac2D/D and hir1D/D cac2D/D mutants are resistant to nicotinamide. CAF-1 plays a major role in maintaining cell types as the cac2D/D mutant exhibited increased switching frequencies in both directions, and switches at a high frequency to opaque in response to nicotinamide. Like the rtt109D/D mutant, the hir1D/D cac2D/D double mutant is defective in maintaining the opaque cell fate, blocks nicotinamide induced opaque formation, and the defects are suppressed by ectopic expression of the master white-opaque regulator Wor1, suggesting an overlapping function of CAF-1 and HIR in epigenetic regulation cell fate determination in a H3K56 acetylation dependent manner.

Epigenetic Regulation of Subtelomeric Gene Noise in *Candida albicans*. Matthew Z Anderson, Joshua A Baller, Lauren J Wigen, Judith Berman. Genetics, Cell Biology and Development, Univeristy of Minnesota, St Paul, MN.

Candida albicans grows within a wide range of fluctuating host niches, and the ability to rapidly adapt enhances its success as a commensal and as a pathogen. The recently expanded telomere-associated (*TLO*) gene family consists of fourteen expressed members in *C. albicans*. Each *TLO* gene encodes a paralog of a single Mediator complex component. Thirteen expressed *TLOs* are located at the chromosome ends as the most telomere-proximal open reading frame. Individual *TLO* expression at both the transcript and protein level was extremely noisy. Noise originated from single cell variability in *TLO* expression due to intrinsic factors. Deletion of chromatin modifying enzymes that function in subtelomeric silencing abolished *TLO* noise, as did ectopically expressing a *TLO* from an internal locus. Conversely, transcriptional variation of a low noise gene increased significantly when ectopically expressed in the subtelomere. Interestingly, deletion of the Mediator component *MED3*, which inhibits Tlo from incorporating into Mediator, also drastically reduced *TLO* noise and supports an autoregulatory mechanism for *TLO* noise. These data suggest subtelomeric chromatin structure regulates *TLO* gene noise through the action of chromatin modifiers and Mediator. We propose that *TLO* noise is beneficial to *C. albicans* by producing heterogeneous cell populations that incorporate different Tlo proteins in Mediator, producing a range of transcriptional profiles in the population that allows some cells to survive in altered environmental conditions.

Chromatin regulation of genome stability. Zachary A. Lewis. Department of Microbiology, University of Georgia, Athens, GA.

Genome instability results from defective DNA replication or repair and is associated with human diseases such as cancer. Chromatin structure impacts virtually all DNA-templated processes in the nucleus, including replication and repair. To identify new chromatin factors that are required for genome stability, we screened the Neurospora knockout collection for strains that are sensitive to the DNA damaging agent methyl methanesulfonate (MMS). The primary screen uncovered over 500 MMS-sensitive knockout strains, including knockouts of putative regulators of chromatin structure. We are currently testing this group of knock out strains for sensitivity to other agents that induce DNA damage. We have also initiated molecular analyses of newly identified regulators of genome stability. Our current progress will be summarized.

Opposing activities of the HCHC and DMM complexes maintain proper DNA methylation in *Neurospora crassa*. Shinji Honda[1,2], Eun Yu[1], Eric Selker[1]. 1) University of Oregon, Institute of Molecular Biology, Eugene, OR; 2) University of Fukui, Life Science Unite, Fukui, Japan.

Proper regulation of heterochromatin and DNA methylation is critical for the normal function of cells. We show that heterochromatin and DNA methylation are faithfully controlled in Neurospora by opposing activities of the silencing complex HCHC and the anti-silencing complex DMM. The workings of these two complexes were investigated. HCHC consists of four proteins, the two chromo domain proteins HP1 and CDP-2, the histone deacetylase HDA-1 and the AT-hook motif protein CHAP. We found that histone deacetylase activity is critical for HCHC function but the H3K9me3 binding activity of the CDP-2 chromo domain is not. Instead, CDP-2 serves as an essential bridge between HP1 and HDA-1. CHAP interacts directly with HDA-1, binds in a methylation-independent way to the A:T-rich DNA that forms the cores of methylated regions and is important for stable association of HDA-1 with chromatin. HCHC is involved in the spreading of DNA methylation in dmm mutants. The DMM complex consists of a presumed histone demethylase, DMM-1, plus DMM-2, which is characterized by a fungal-specific $Zn(II)_2Cys_6$ DNA-binding domain ("Zn-Cys"). We found that DMM-2 strongly binds to DNA from euchromatin/heterochromatin junctions, thereby promoting the stable association of DMM-1 at the edge of heterochromatin domains to prevent aberrant spreading of DNA methylation.

Wednesday, March 13 3:00 PM–6:00 PM
Kiln

Genomics and Mycorrhizae

Co-chairs: Anders Tunlid and Tom Bruns

The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq. A. Kohler[1], E. Tisserant[1], E. Morin[1], C. Veneault-Fourrey[1], S. Abba[2], F. Buscot[3], J. Doré[4], G. Gay[4], M. Girlanda[2], S. Herrmann[3], T. Johansson[5], U. Lahrmann[6], E. Martino[2], S. Perotto[2], M. Tarrka[3], A. Tunlid[5], A. Zuccaro[6], I. Grigoriev[7], F. Martin[1]. 1) Lab of Excellence ARBRE, Tree-Microbes Department, INRA-Nancy, Champenoux, France; 2) Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino,Torino, Italy; 3) Department Soil Ecology, UFZ Centre for Environmental Research Leipzig-Halle Ltd., Halle, Germany; 4) Ecologie Microbienne UMR CNRS 5557, USC INRA 1193, Universite Claude-Bernard LYON 1, Villeurbanne, France; 5) Microbial Ecology, Lunds University, Lund, Sweden; 6) Max-Planck Insitute for Terrestrial Microbiology, Marburg, Germany; 7) DOE Joint Genome Institute, Walnut Creek, California, USA.

Genome and transcriptome analyses of *Laccaria bicolo*r and *Tuber melanosporum* (Martin *et al.*, 2008, 2010) revealed that the ectomycorrhizal symbiosis probably developed several times during evolution by generating different 'symbiosis molecular toolkits'. In *L. bicolor* a large set of small-secreted proteins acts as putative effectors but not in T. *melanosporum*, while the up-regulation of transporter-coding genes seems to be a common feature of both interactions. To better understand the evolutionary origin of mycorrhizal symbiosis and to elucidate the molecular mechanisms involved, a large sequencing project of species from different taxa, phylogenetic clades and symbiotic lifestyles (ectomycorrhizae, ericoid and orchid mycorrhizae) was started in 2011 by the Joint Genome Institute and the mycorrhizal genome initiative. To identify and to compare symbiosis-regulated genes large scale Illumina transcriptome sequencing of mycelium and mycorrhizal roots from *Paxillus involutus, Piloderma croceum, Hebeloma cylindrosporum, Sebacina vermifera, Tulasnella calospora* and *Oidiodendron maius* was performed. Small-secreted proteins, transporters, CAZymes but also many lineage specific proteins were among the highly up-regulated transcripts.

Martin, F., Aerts, A., Ahrén, D., Brun, A., Duchaussoy, F., Kohler, A., et al. 2008. The genome sequence of the basidiomycete fungus Laccaria bicolor provides insights inot the mycorrhizal symbiosis. Nature 452 :88-92

Martin, F., Kohler, A., Murat, C., Balestrini, R., Coutinho, P.M., Jaillon, O., Montanini, B., et al. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464 :1033-1038.

Transposable element dynamics in the *Amanita*: insights on the evolution of genome architecture accompanying the transition from saprotrophic to ectomycorrhizal ecologies. Jaqueline Hess[1], Inger Skrede[2], Anne Pringle[1]. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Microbial Evolution Research Group, Department of Biology, University of Oslo, Oslo, Norway.

Transposable elements (TEs) form an integral structural part of the genomes of many higher Eukaryotes. Their ability to proliferate independently and into a large number of copies can lead to extensive amounts of repetitive DNA that is of no obvious benefit to the host. At first thought to be relatively underrepresented in Fungi, genome sequencing over the last decade has led to the discovery of many fungal genomes that are densely populated with TEs. Among those are the genomes of the ectomycorrhizal (ECM) fungi *Laccaria bicolor* (around 30% TE) and *Tuber melanosporum* (around 60% TE) as well as a number of fungal pathogens, including *Puccinia graminis* and *Melamspora larici-populina* (both around 45% TE). The high TE content in these species, especially when compared to saprotrophic fungal species, suggests an association between symbiotic ecology, both mutualistic and antagonistic, and the ability of TEs to invade and persist in their genomes. However, the mechanisms for this are currently not well understood. In order to assess whether high TE content is a feature of other ECM species and to get a more detailed picture of TE content changes around the transition from free-living to ECM ecology, we have sequenced the genomes of five members of the genus *Amanita*: three ECM species and two saprotrophs, as well as the saprotrophic outgroup *Volvariella volvacea*. Using the draft genome assemblies, we have developed methodology to estimate TE content from short-read data and examine changes therein within quantitative and phylogenetic frameworks. Overall, we find no direct relationship between ECM status and increased TE content in the *Amanita* but instead discover patterns that suggest population genetics to be a strong driver of TE content. We will discuss our findings with respect to the influence of TEs in the evolution of genome architecture around the origin of ECM symbiosis.

Broad compatibility in the root endophyte *Piriformospora indica* is associated with host-adapted colonization strategies. Urs Lahrmann, Yi Ding, Alga Zuccaro. Organismic Interactions, MPI Marburg, Marburg, Germany.

Their host range defines plant associated fungi as either specialists, which are adapted to one or few distinct hosts, or generalists who are able to thrive in highly variable host environments. Specialists and their hosts are in an evolutionary arms race that leads to the development of weapons perfectly tailored to the respective host. Conversely, broad-host range species must evolve adaptations to cope with a plethora of different host-associated signals and host-specific defense mechanisms. The evolutionary force, in this case, drives the expansion and diversification of the fungal arsenal and the host-adapted gene expression to better suite different plants. The mechanisms underpinning broad compatibility in root symbiosis are largely unexplored. The generalist root endophyte *Piriformospora indica* that stimulates growth, alleviates salt stress and induces systemic resistance to pathogens in different hosts can establish a long lasting interaction with the roots of barley and Arabidopsis, two morphologically and biochemically very distinct plants. We show here that in these two hosts, root colonization proceeds very differently. While in Arabidopsis the fungus establishes and maintains biotrophic nutrition within living epidermal cells, in barley the symbiont undergoes a nutritional switch to saprotrophy that is associated with the production of secondary thinner hyphae (SH) in dead cortex cells. Consistent with a diversified trophic behavior, genome-wide expression profiling revealed a strong induction of genes encoding cell wall degrading enzymes and nutrient transporters in barley but not in Arabidopsis at a late colonization stage. In particular small secreted proteins (SSPs < 300 amino acids) known as effectors have been shown to facilitate colonization by manipulating host defense and reprogramming plant metabolism during symbiosis. Expression of *P. indica* genes encoding SSPs was induced in both hosts at different symbiotic stage, but the majority of these SSPs were either Arabidopsis or barley responsive with the larger number expressed during biotrophy in Arabidopsis and during saprotrophy in barley. Our study reveals that broad compatibility in root endophytes requires strong phenotypic plasticity and the expression of alternative lifestyle strategies in a host-dependent way.

Examining the saprotrophic ability of ectomycorrhizal fungi using genomics, transcriptomics and spectroscopy. Anders P V Tunlid. Dept Microbial Ecology, Ecology, Lund, Sweden.

A large part of the nitrogen in forest soils is found in recalcitrant organic matter- protein complexes. Ectomycorrhizal fungi are thought to have a key role in the decomposition and mobilization of nitrogen from such complexes. The knowledge on the functional mechanisms of these processes, and how they are regulated by carbon from the host plant and the availability of more easily available forms of nitrogen sources are limited. We examined how the ECM fungus *Paxillus involutus* degrade organic litter material using spectroscopy and transcriptome profiling. The fungus partially degraded polysaccharides and modified the structure of polyphenols. The observed chemical changes and the expressed transcriptome were consistent with a hydroxyl radical attack, involving Fenton chemistry similar to that of brown-rot fungi. The set of enzymes expressed by *P. involutus* during the degradation of the organic matter was similar to the set of enzymes involved in the oxidative degradation of wood by brown-rot fungi. However, *P. involu*tus lacked transcripts encoding extracellular enzymes needed for metabolizing the released carbon. Further experiments have shown that the decomposition and assimilation of nitrogen from organic litter material are triggered by adding glucose. Addition of easily available forms of nitrogen (i.e. ammonium) had minute effects on these processes. Experiments and comparative genomics demonstrate that the saprotrophic activity of *P. involutus* has been reduced to a radical-based biodegradation system that can efficiently disrupt the organic matter-protein complexes and thereby mobilize the entrapped nutrients.

Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots. Nils OS Högberg[1], Anna Rosling[1], Annegret Kohler[2], Martin Francis[2], Stenlid Jan[1]. 1) Department of Forest Mycology, BioCenter, SLU, Uppsala, Sweden; 2) INRA, Nancy, France.

Recently it has been shown, with a Comparative genomic perspective, that brown rot and mycorrhiza fungi have evolved from white rot ancestors. Wood is a composite material composed of lignin, cellulose and hemicellulose. White rot fungi are able to degrade all of these components with a combination of carbohydrate active and oxidative enzymes. During the course of evolution brown rot and mycorrhiza have lost most of the genes in these gene families. Nevertheless, brown rot fungi are efficient wood decomposers that degrade cellulose and hemicellulose by means of hydroxyl radical production and remaining carbohydrate active enzymes. The family Boletales includes both brown rot fungi and mycorrhiza and it is tentative to speculate that there has been a parallel evolution of these ecological strategies. Here we test the effect of infecting pine roots with the brown rot fungus Serpula lacrymans. The interaction was neutral since plant growth was not stimulated but not reduced either. The fungus formed a mantle around the pine roots but not the Hartig net that is typical for ectomycorrhiza. Fungal gene expression was compared with the wood decay transcriptome. 1250 genes were more than two-fold upregulated compared to a glucose medium control. A large proportion of the upregulated genes (62 %) are unknown. Carbohydrate active genes represent only 3% of this gene set and genes with oxidoreductase activity, including monoxygenases represent 4% of the upregulated genes. This is considerably lower compared to saprotrophic growth on wood where carbohydrate active enzymes accounted for 26% and oxidative enzymes for 19% which dominated the gene expression on wood. Gene expression for genes involved in transportation was about the same, around 10% in this experiment and under wood decomposition. Several genes that indicate an interaction with a host were also upregulated. In conclusion, gene expression was markedly different between a glucose medium, wood decomposition and growth on pine roots. This may be a signal of symbiosis, the effect on pine seedling growth was neutral. Thus we cannot conclude if the interaction is beneficial or negative to the host.

Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis. Stephen J. Mondo, Teresa E. Pawlowska. Plant Pathology, Cornell University, Ithaca, NY.

Many eukaryotes interact with heritable endobacteria to satisfy diverse metabolic needs. Of the characterized fungal-bacterial endosymbioses, the association between Gigasporaceae (Glomeromycota) and Ca. Glomeribacter is one of the best described. Glomeribacter is a member of the Burkholderia lineage of b-proteobacteria, and was shown previously to represent one of the few cases of an ancient, long-term non-essential endosymbiont. In order to further explore what adaptations have taken place to shape this unique bacterial lifestyle, we have sequenced three Glomeribacter genomes and developed a computational pipeline to compare across bacteria engaging in different lifestyles using genome wide patterns of mutation accumulation. We used PAML to identify gene orthologs that exhibited both over-accumulation and under-accumulation of amino acid substitutions and then used these data to compare across taxa at the level of functional gene categories. We found that bacteria can be grouped by lifestyle using this approach. Glomeribacter, as expected, appears most similar to other potentially long-term non-essential endosymbionts. Therefore, we were able to exploit the differences in mutation accumulation patterns between these taxa to identify processes, which may be relevant within the particular interaction between Glomeribacter and its host. While several of these processes, including vitamin synthesis and amino acid transport, have been identified previously, we additionally discovered features related to lipid biosynthesis and energy metabolism to be of potential importance for this symbiosis. Interestingly, genes exhibiting an under-accumulation of nonsynonymous substitutions (indicative of purifying selection) in Glomeribacter tend to be involved in recombination, cell division, and ribosome maintenance. While these processes are typically fast evolving in endosymbiotic organisms, they may represent features that increase the stability of Glomeribacter in their fungal host population and increase their resilience to genetic drift. We speculate that these processes are unique to the Glomeribacter-Glomeromycota symbiosis and could partially explain why Glomeribacter has been successful as a non-essential endosymbiont for over 400 million years.

A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon. Alija Mujic, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Species of Rhizopogon are EM symbionts of trees in family Pinaceae and produce basidiospores within hypogeous false truffles that are dispersed by mycophagous mammals. All known members of R. subgenus Villosuli form obligate EM relationships with Pseudotsuga spp. (Douglas Fir) and are the only members of the genus known to possess this host association. R. vesiculosus, along with its cryptic sister species R. vinicolor, possess a sympatric distribution where sampled within the range of their host tree, P. menziesii. While the sporocarp and EM morphology of these fungi may be highly similar; they possess striking life history differences with R. vesiculosus producing larger vegetative genets and displaying greater population structure at both local and landscape scales. We have sequenced the genome of R. vesiculosus using dikaryotic tissue and a whole genome shotgun sequencing approach on the Illumina HiSeq platform. De novo assembly of the genome was performed using VELVET 1.19 and gene predictions were made using AUGUSTUS with Laccaria bicolor as a training model. The draft genome assembled to a total length of 46 Mb in 6700 contigs with an N50 of 26,783, a maximum contig size of 446,818 bp, and 12,604 predicted genes. Here we characterize the mating system of R. vesiculosus, which possesses both an A-locus encoding a heterodimer transcription factor, as well a B-locus encoding transmembrane pheromone receptors and pheromone precursor genes. We present comparisons of the mating system of R. vinicolor and its similarities to other members of Boletales (e.g., Serpula) and differences with Agaricales (e.g., Laccaria). Due to the dikaryotic nature of the genome sequence produced for R. vesiculosus, single nucleotide polymorphisms (SNPs) can be observed and used to characterize allelic variation. SNPs observed in protein coding regions of both MAT loci indicate that R. vesisculosus is likely heterothallic. We have also characterized heterozygosity across the whole genome in order to identify hypervariable regions. This genome will allow for comparative analysis of gene content, mating type system with other Basidiomycota and, ultimately, for population/species-level genomic studies within Rhizopogon.

Metatranscriptomic analysis of ectomycorrhizal root clusters in *Pinus taeda*: new methodologies for assessing functional gene expression *in situ*. H.-L. Liao[1], Y. Chen[2], T. D. Bruns[3], K. G. Peay[4], J. W. Taylor[3], S. Branco[3], J. M. Talbot[4], R. Vilgalys[1]. 1) Department of Biology Duke University, Durham, NC; 2) School of Medicine, Duke University, Durham, NC; 3) Department of Plant and Microbial Biology, UC-Berkeley, Berkeley, CA; 4) Department of Biology, Stanford University, Stanford, CA.

A highly diverse community of ectomycorrhizal (ECM) fungi are known to associate with members of the genus *Pinus*. Less is known about how diverse fungal communities affect functional diversity within ECM roots. Here we present an optimized method for metatranscriptomic analysis of the ECM-pine root interaction in a natural system. RNA was purified using a CTAB method from individual ECM root clusters collected at varying spatial scales across the distribution range of *P. taeda*, and sequenced using Illumina HiSeq technology. About 35 million qualified reads were obtained. Sequences were initially assembled using reference based mapping (Bowtie) to sort the reads that represent rRNA from fungal and bacterial species. Reads from divergent regions (D1-D2) of fungal LSU rRNA were used to identify dominant ECM and other fungal community members. Subsequently, *P. taeda* genes and functional genes of dominant fungal species were sorted using public cDNA databases. The Trinity package was used for *de novo* assembly of un-mapped reads (mostly fungal genes). Blastx and Go packages were used for gene annotation. A typical ECM root cluster was found 45% *P. taeda* genes, 3% fungal rRNA, 0.05% bacterial 16S rRNA, 30% fungal functional genes, 10% unknown sequences, and 12% unassembled reads. Analysis of D1-D2 LSU sequences confirmed that a single ECM fungal species usually dominates individual root clusters. *De novo* assemblies of fungal genes yielded 120 thousand contigs from 10 million reads representing 90 thousand unique genes with highly similarity to known ECM fungi. Functional analysis revealed that most of the transcripts recovered were involved with translation, protein degradation, heat shock, superoxide metabolism, electron transfer, signaling, and C/N metabolism. Highly expressed transcripts recovered from *Piloderma*, which was abundant in our samples, included genes encoding a wide array of metabolic enzymes: chitosanase, phosphatase, glutamine synthetase, terpene synthases, b-glucanase; transporters for P+ and oligopeptides; cell signaling: calmodulin, cAMP-regulated phosphoprotein (Igo1); C/N related genes: lectin, cross-pathway control (cpc1); as well as several genes with unknown function. Future studies will seek to address how ECM metatranscriptomes change in response to different *Pinus* hosts and across different spatial scales.

Wednesday, March 13 3:00 PM–6:00 PM
Nautilus

Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism

Co-chairs: Richard Wilson and Ronald de Vries

The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhiza. Carl R. Fellbaum[1], Emma W. Gachomo[1], Gary D. Strahan[2], Philip E. Pfeffer[2], E. Toby Kiers[3], Heike Bücking[1]. 1) Biology and Microbiology, South Dakota State University, Brookings, SD; 2) Agricultural Research Service, Eastern Regional Research Center, US Department of Agriculture, Wyndmoor, PA; 3) Department of Ecological Science, Vrije Universiteit, Amsterdam, The Netherlands.

Arbuscular mycorrhizal (AM) fungi can substantially contribute to host plant nitrogen (N) nutrition in exchange for carbon (C). We studied the effect of C supply on fungal N uptake and transport in the AM symbiosis via ^{15}N labeling, enzymatic assays and qPCR analysis of fungal genes putatively involved in N metabolism. We found that an increase in C supply stimulated ^{15}N transport and increased the enzymatic activity of arginase and urease in the intraradical mycelium (IRM). The fungus responded to an increase in the C supply with an upregulation of genes involved in N assimilation and arginine biosynthesis, but with a downregulation of a fungal urease in the extraradical mycelium (ERM). The effect on fungal gene expression in the IRM was relatively small, but genes involved in arginine biosynthesis were downregulated by an increase in C availability. The results indicate that C from the host triggers N uptake by the AM fungus, the conversion of N into arginine in the ERM, the transport of arginine to the IRM and subsequent breakdown of arginine via the catabolic arm of the urea cycle. When the fungus had access to a C supply independent from the host ^{15}N transport was reduced and a change in the gene expression pattern indicated that the fungus changed its nutrient allocation strategy when the fungus was less dependent on the host for its C supply. In a common mycelial network, the AM fungus *Glomus aggregatum* transported more N to a more photosynthetically active plant when given the choice between a shaded versus a non-shaded plant host. The results indicate that AM fungi are able to distinguish between hosts differing in their carbon supply and that carbon is an important trigger for fungal nitrogen uptake and transport in the AM symbiosis.

Mechanisms of adaptation to host rice cells by the blast fungus. Jessie Fernandez, Richard A. Wilson. Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68516, USA.

To infect rice, the devastating blast fungus *Magnaporthe oryzea* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. How the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf, what controls the genetic reprogramming necessary to produce infectious hyphae, and how it acquires nutrient during biotrophic in planta growth is poorly understood. *M. oryzae's* trehalose-6-phosphate synthase 1 (Tps1) enzyme integrates carbon and nitrogen metabolism in the fungal cell to regulate virulence via a novel NADPH-dependent genetic switch. Loss of Tps1 function results in D*tps1* strains that can form functional appressoria and penetrate the rice surface but fail to grow beyond the first infected cell. Impaired invasive growth of D*tps1* strains is due to loss of glucose sensing, inactivation of the NADPH-dependent genetic switch, and altered carbon assimilation. Moreover, NADPH-requiring antioxidation systems are shut down in D*tps1* strains, rendering them hypersensitive to oxidative stress. Taken together, we discuss here how, using classical and high-throughput reverse genetics, we are exploring the dynamics of this critical NADPH-dependent genetic switch to understand how *M. oryzae* controls infectious hyphal development during biotrophy, how it responds to and acquires nutrient from the host, and how these processes are integrated to allow successful colonization of rice cells.

Similar is not the same: Differences in the function of the (hemi-) cellulolytic regulator XlnR (Xlr1/Xyr1) in filamentous fungi. Sylvia Klaubauf[1*], Hari Mander Narang[1], Evy Battaglia[2], Tetsuo Kobayashi[3], Kurt Brunner[4], Astrid R. Mach Aigner[4], Robert L. Mach[4], Ronald P. de Vries[1,2]. 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; 2) Microbiology, Utrecht University, Utrecht, Netherlands; 3) Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya-shi, Aichi, Japan; 4) Institue of Chemical Engineering, Research Area Biotechnology and Microbiology, Working Group Gene Technology, Vienna, Austria.

The (hemi-) cellulolytic transcriptional activator XlnR (Xlr1/Xyr1) is a major regulator in fungal xylan and cellulose degradation as well as in the utilization of D-xylose via the pentose catabolic pathway. XlnR homologs are commonly found in filamentous ascomycetes and often assumed to have the same function in different fungi. However, a comparison of the saprobe *Aspergillus niger* and the plant pathogen *Magnaporthe oryzae* showed different phenotypes for deletion strains of XlnR. In this study wild type and *xlnR/xlr1/xyr1* mutants of six fungi were compared: *Fusarium graminearum*, *M. oryzae*, *Trichoderma reesei*, *A. niger*, *Aspergillus nidulans* and *Aspergillus oryzae*. The comparison included growth profiling on relevant substrates and detailed analysis of protein profiles of extracellular enzymes as well as extracellular enzyme activities. The resulting data demonstrated significant differences in the influence of XlnR and its orthologs on plant polysaccharide degradation by these fungi. For example, in *A. niger* cellulolytic enzymes, such as cellobiohydrolase and b-glucosidase are strongly down-regulated in the mutant strain, whereas this is not the case for the other two *Aspergillus* species. Moreover, in *A. oryzae* the L-arabinose releasing enzyme a-arabinofuranosidase is clearly regulated by AoXlnR, whereas this enzyme is known to be under control of another regulator, AraR, in *A. niger* and not affected by XlnR. In contrast, *M. oryzae* Xlr1 does not significantly affect enzyme activities in this study. Based on extracellular protein profiles, disruption of Xyr1 results in the disappearance of only some bands in *F. graminearum*, while nearly all bands disappear in *T. reesei Dxyr1*. This comparison emphasizes the functional diversity of a fine-tuned (hemi-) cellulolytic regulatory system in filamentous fungi, which might be related to the adaptation of fungi to their specific biotopes.

Regulating the *Aspergillus nidulans* global nitrogen transcription factor AreA. Richard B. Todd. Department of Plant Pathology, Kansas State University, Manhattan, KS.

Nitrogen nutrient utilization genes are regulated in *Aspergillus nidulans* by the GATA DNA-binding transcription activator AreA. The transcriptional activity of AreA is highly regulated by multiple mechanisms including autogenous transcriptional regulation, differential *areA* transcript stability, interaction of AreA with the corepressor NmrA, and repression by the negative-acting GATA factor AreB. In addition, AreA shows regulated nuclear accumulation. AreA accumulates in the nucleus specifically during nitrogen starvation, and is rapidly exported to the cytoplasm upon addition of nitrogen nutrients to nitrogen-starved cells. I will focus on recent developments in our understanding of AreA nuclear import and nuclear export, the key control points of regulated AreA nuclear accumulation. We have shown that the six conserved nuclear localization signals (NLSs) in AreA show redundancy and collaborate to mediate nuclear import. In contrast, a single CrmA exportin-dependent nuclear export signal (NES) in AreA is required for nuclear export. We have shown that fusion of the AreA NES to a constitutively nuclear protein confers nucleocytoplasmic localization and a loss of function phenotype. We have exploited this phenotype to select mutants defective in the AreA-CrmA interaction.

Transcriptional analysis of oxalate degradation in the white rot basidiomycete *Dichomitus squalens*. Miia R. Mäkelä, Johanna Rytioja, Outi-Maaria Sietiö, Sari Timonen, Annele Hatakka, Kristiina Hildén. Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

Basidiomycetous white rot fungi are the most efficient degraders of lignocellulose with a unique ability to mineralize the recalcitrant lignin polymer. Lignocellulose decay involves a complex enzymatic system, but is also suggested to be promoted by the fungal secretion of oxalic acid. White rot fungi synthesize oxalate as a metabolic waste compound and typically secrete it to their environment in millimolar quantities. As oxalate is a toxic compound, regulation of its intra- and extracellular concentration is extremely crucial for fungi and also for lignocellulose degradation since high oxalate levels are shown to inhibit the decomposition reactions. Therefore, specific oxalate-converting enzymes, namely oxalate decarboxylases (ODCs) that work in conjunction with formate-degrading formate dehydrogenases (FDHs), are recognized as key fungal enzymes in lignocellulose decay. *Dichomitus squalens* is a white rot fungus that degrades effectively all the wood polymers, i.e. cellulose, hemicelluloses and lignin, and secretes oxalic acid during its growth on wood. The genome of *D. squalens* harbours 5 putative ODC and 3 putative FDH encoding genes, while these numbers differ in other fungi based on comparative genomics. In order to enlighten the roles of the multiple oxalic-acid catabolising enzymes of *D. squalens*, the expression of the *odc* and *fdh* genes was followed with quantitative real-time RT-PCR when the fungus was grown on its natural substrate, i.e. Norway spruce (*Picea abies*) wood. In addition, the effect of organic acid (oxalic acid) and inorganic acid (HCl) supplementation on the relative transcript levels of the oxalate-catabolizing genes was examined in the submerged liquid cultures of *D. squalens*. The results show for the first time the sequential action of ODC and FDH at the transcript level in a white rot fungal species. The constitutive expression of *odc1* suggests the pivotal role of the corresponding enzyme during the growth of *D. squalens* on wood. In addition, the strong upregulation of the transcription of *odc2* in oxalic-acid amended cultures indicates the distinct roles of individual ODC isoenzymes.

TOR-mediated control of virulence functions in the trans-kingdom pathogen *Fusarium oxysporum*. Gesabel Y. Navarro Velasco, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain.

Infectious growth of fungal pathogens is controlled by environmental cues, including nutrient status. The soilborne fungus *Fusarium oxysporum* produces vascular wilt disease in more than a hundred different crop species and can cause lethal systemic infections in immunodepressed humans. Previous work showed that the preferred nitrogen source ammonium causes repression of infection-related processes in *F. oxysporum* that could be reversed by rapamycin, a specific inhibitor of the conserved protein kinase TOR. Here we generated mutations in upstream components that should result in constitutive activation of TOR, including null mutants in tuberous sclerosis complex 2 (TSC2), a small GTPase that represses TOR activity, as well as strains expressing a dominant activating allele of the small GTPase Rag (*ragA*^{Q86L}), an activator of TOR. The D*tsc2* mutants and, to a minor extent, the *ragA*^{Q86L} strains showed defects in hyphal growth and colony morphology on several amino acids, as well as decreased efficiency in cellophane penetration and vegetative hyphal fusion. These phenotypes were exacerbated in D*tsc2ragA*^{Q86L} double mutants and could be reversed by rapamycin, suggesting that they are caused by hyperactivation of TOR. The mutants caused significantly lower mortality on tomato plants and on larvae of the animal model host *Galleria mellonella*. These results suggest that TOR functions as a negative regulator of fungal virulence on plant and animal hosts.

Transcriptional regulation of peptidases and nitrogen transporters during the assimilation of organic nitrogen by the ectomycorrhizal fungi *Paxillus involutus*. Firoz Shah[1], Francois Rineau[2], Tomas Johansson[1], Anders Tunlid[1]. 1) Microbial Ecology Group, Department of Biology, Lund University, SE-22362, Lund, Sweden; 2) Centre for Environmental Sciences, Hasselt University, Building D, Agoralaan, 3590 Diepenbeek, Limburg, Belgium.

Proteins and amino acids form a major part of the organic nitrogen (N) sources in soils. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the role of ectomycorrhizal extracellular peptidases and amino acid transporters in the degradation, uptake and transfer of various protein sources (BSA, Gliadin and pollen) as well as of plant litter material using the ECM model fungus *Paxillus involutus*. During N-deprived conditions, all substrates induced secretion of peptidase activities. The activity had acidic pH optimum (2.3-3.0), and it was mainly due to aspartic peptidases and with minor contribution of metallo and serine peptidases. The activity was partly and temporarily repressed by low concentrations of ammonium (1mg/L). Transcriptional analysis showed that *P. involutus* expressed a large array of proteins and enzymes involved in the assimilation of organic N including peptidases, N-transporters and enzymes of the N-metabolism. Extensive *in-silico* analysis revealed the presence of genes encoding 312 peptidases, 129 N transporters and 284 enzymes involved in amino acid metabolism. Out of these, 89 peptidases and 37 N-transporters and 109 amino acid metabolism enzymes encoding genes were significantly upregulated during organic N assimilation. The genes were encoding a variety of secreted (23) and non-secreted (20) peptidases which were differentially expressed depending on the medium with the highest expression of the aspartic and metallo peptidases. Apart from the YAAH/ATO family, upregulated genes were found in all the other families of transporters for amino acids, oligopeptides, ammonium, urea and allantoate/allantoin. The results shows that the expression levels of peptidases and transporters in *P. involutus* are coordinately regulated during the assimilation of organic N sources.

Regulation of glycolysis and gluconeogenesis by antisense transcription in *Aspergillus nidulans*? Michael Hynes[1], Koon Ho Wong[2], Sandra Murray[1]. 1) Dept Gen, Univ Melbourne, Parkville, Victoria, Australia; 2) Dept. of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA.

The last step in glycolysis is carried out by pyruvate kinase, encoded by *pkiA*, converting phospho-enol-pyruvate to pyruvate for metabolism to oxaloacetate and acetyl-CoA. The key step in gluconeogenesis is conversion of oxaloacetate to phospho-enol-pyruvate by PEP carboxykinase, encoded by *acuF*. Simultaneous activity of these enzymes would generate a nasty futile cycle. A number of observations suggests that control of the expression of these two genes involves activation of sense transcription and negative control by activation of antisense transcription. For *pkiA*, ChIP studies have found binding of the gluconeogenic activators AcuK and AcuM and of the acetate dependent FacB activator in the downstream region. Cognate binding sites are conserved in filamentous ascomycetes. RNA Seq, polII ChIP and RT-PCR analysis indicates antisense transcription during growth on acetate or proline as carbon sources. Old data (de Graaf, van den Broek, Visser; Cur. Genetics 13: 315, 1988) showed that transformation of a construct lacking these 3' sites resulted in inappropriate *pkiA* expression on acetate. In response to growth on gluconeogenic carbon sources, the *acuF* gene is activated by AcuK and AcuM binding to sites in the 5' upstream region. Studies with an *acuF-lacZ* gene fusion indicate positive control by AcuK and AcuM but a loss of the glucose repression observed in Northerns suggesting negative regulation acting via 3' sequences in response to growth on glycolytic carbon sources. Support for this is provided by transcription studies. Modulation of the balance between the opposing activities of these two gene products is proposed to result from transcriptional interference involving collision of RNA polymerase molecules.

Wednesday, March 13 3:00 PM–6:00 PM
Scripps

Education, Outreach, and Professional Development

Co-chairs: Steven Denison and Mimi Zolan

Centrosome-Nuclear Disconnect Creates Mitotic Chaos in a Closed Mitosis System. Michael Koonce, Irina Tikhonenko. Translational Medicine, Wadsworth Center, NYS Department of Health, Albany, NY.

In many fungi, protists, and unicellular algae, cells divide via a mitotic mechanism that functions within a closed nuclear compartment. Closed mitosis requires tight coordination between the centrosome and nucleus to ensure a smooth transition from cytoplasmic to nuclear activities. In many cases, the centrosome/spindle pole body is directly embedded into the nuclear envelope, an arrangement that facilitates this transition. However in other organisms (e.g. Cryptococcus, S. Pombe, Dictyostelium), the centrosome is simply attached to the cytoplasmic side of the nucleus during interphase, gaining access to the intranuclear volume only during mitosis when this organelle builds the spindle. We have identified a kinesin motor protein (Kif9) that is responsible for maintaining the centrosome-nucleus interaction in Dictyostelium. In the absence of Kif9, centrosomes separate from nuclei and the two organelles move independently. We used this null mutant to examine what happens if centrosomes fail to engage nuclei when cells enter mitosis and to examine interactions in multi-nucleated arrangements. We find that centrosomes replicate and separate in the cytosol, but do not form a visible spindle apparatus. Nuclei that lack centrosomes import tubulin, but also fail to develop a spindle apparatus. Replicated daughter centrosomes are able to integrate into the nuclear envelope and can go on to form monopolar spindles. All centrosomes (nuclear or cytoplasmic) trigger cytokinesis activity. Moreover, the nuclear envelope is promiscuous and can dock multiple centrosomes if they are available. In these cases, multipolar or multiple independent spindles can arise which lead to aneuploid nuclear products. Our work illustrates the significance of maintaining a one centrosome-one nucleus relationship to ensure proper chromosome segregation. This is particularly important in multinucleated syncytia where unpaired activities would result in multiple combinations of centrosome-nuclear engagements. In addition, a firm coupling between these two organelles links nuclei to microtubule force generating machinery that is crucial for nuclear transport and positioning. We will further present a model for how the Kif9 kinesin functions to maintain centrosome-nuclear paring. For our work, we gratefully acknowledge support from the NSF (MCB-1051612).

Using Fungal Barcoding to Introduce Non-science Majors to Scientific Research. Claire Burns. Washington & Jefferson College Washington, PA.

Non-scientists, even those with an interest in biology, tend to have a poor understanding of fungi; one example is the perception that mushrooms are "some kind of weird plant". This lack of awareness regarding fungi and their importance allows non-majors students to enter the classroom without preconceptions or expectations. Fungi provide an excellent starting point to introduce students to broader biological themes such as cell biology, diversity of life, evolution, conservation, and molecular biology. In addition, the relative paucity of species classification for fungi when compared with other kingdoms provides an opportunity for non-majors students to engage in primary research projects. In a liberal arts college class entitled "'Shrooms", students collect mushrooms in the field, cultivate the fungi, extract DNA, and identify the species using DNA barcoding. Fungal identifications and sequences can contribute to ongoing fungal barcoding efforts. In future classes, students will expand this research to include identification of soil-borne fungi at a field station located close to gas-fracking operations.

ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis. Gita Bangera[1], Andrea Gargas[2]. 1) Bellevue College, Bellevue, WA, USA; 2) Symbiology LLC, Middleton, WI, USA.

ComGen (Community College Genomics Research Initiative) teaches students the skills of self-directed learning, critical thinking, and analysis. Community college students in this program receive a mini-graduate school experience, following a single requisite course in cell biology. Students work on original research projects, learn to troubleshoot their experiments, organize lab meetings and student journal clubs, and network within the scientific community. In one research track students work with DNA from described fungal collections, learning DNA-based techniques including PCR amplification, DNA sequencing and sequence analysis. Student-gathered sequence information is used to advance identification and phylogenetic results for these collections. With NSF Award DUE #1225857 ComGen (C-ARE): Dissemination, Enrichment and Expansion Project the project will be expanding to community college partners throughout the Seattle/Tacoma region of Washington State.

Wearing two hats: Tips for combining commitments to research and to university-wide initiatives in education. Patricia J. Pukkila. Dept Biol, Univ North Carolina, Chapel Hill, NC.

In an editorial which appeared in the New York Times, Gary Gutting argued that the primary role of universities is to "nourish a world of intellectual culture; that is, a world of ideas, dedicated to what we can know scientifically, understand humanistically, or express artistically" (http://opinionator.blogs.nytimes.com/2011/12/14/what-is-college-for/). At research universities, faculty are expected to make substantial contributions to their disciplines, to society, and to educating students. It is important for faculty to seek pan-university roles, and making acknowledged contributions to changing your campus culture can be deeply satisfying. This session will encourage you to consider how you might productively multitask in ways that can actually benefit your research productivity in addition to improving the intellectual climate on your campus. Supported in part by the HHMI through the Precollege and Undergraduate Science Education Program.

Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread. Virginia K. Hench[1,2], Patricia J. Pukkila[1,2]. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

The HHMI-Future Scientists and Clinicians (HHMI-FSC) fellowship is 1 of 3 components of the HHMI Science Learning Communities program at UNC Chapel Hill. The HHMI-FSC program was designed to foster an intellectual community that empowers high-ability students from low-income backgrounds to engage in biomedical research for 2 summers. Each year, 12 new fellows are matched with mentors in labs spanning a range of biomedical areas. They work fulltime in labs on their own research project and meet weekly as a group to engage in interactive programming that targets skills critical for success in science beyond the bench. One area of emphasis has been the process of inquiry itself. The goal is for students to transition from being a pair of hands executing protocols to active learners invested in their own projects and able to speak with authority about why experiments are performed in particular ways and what conclusions can be drawn from data generated. This starts with coaching students to state the questions that they are trying to answer and think through whether an experimental setup is consistent with what they say they are trying to find out. Assignments and feedback are designed to reinforce this principle. One of the most satisfying aspects of doing science is getting to follow one's own instinctive curiosities and develop the methodologies needed to navigate new terrains. Undergraduates are usually still trying to define their own specific curiosities. Pushing students to describe what they are curious and passionate about is one feasible strategy that can help students identify pursuits that fit their interests and talents. Another successful strategy has been to require returning second year fellows to share science learning experiences via 15-30 minute long talks for their peers. Some took the opportunity to become more immersed in their lab's focus, while others branched into questions like what motivates scientists to work in foreign countries and what has genomic anthropology told us about human evolution. Project aims were developed through conversations between the fellow and instructor. The one constraint was for fellows to organize their presentations around questions. Feedback indicated that presenters benefited from having to give presentations and others enjoyed learning about a broader array of topics.

MOOCs: Education for Everyone. Relly Brandman. Course Operations, Coursera.

Thursday, March 14 3:00 PM–6:00 PM
Merrill Hall

Cool Tools for Fungal Biology

Co-chairs: Miguel Penalva and Kevin McCluskey

The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology. S. E. Baker. Environmental Molecular Sciences Laboratory, Pacific Northwest Natl Lab, Richland, WA.

Tools for analysis of classical and reverse genetic mutants play an important role in fungal biology research. The Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory is a US Department of Energy national user facility. EMSL develops and utilizes cutting edge mass spectrometry, NMR, imaging and computational capabilities to accelerate research in a number of areas. We have used EMSL's mass spectrometry capabilities to characterize glycosylation of secreted proteins of *Aspergillus niger*. In addition, we have explored the use of laser ablation and nano-DESI mass spectrometry for spatial localization of molecules associated with *Trichoderma reesei* mycelium. Finally, spores from wildtype and albino strains of *Aspergillus carbonarius* were characterized using helium ion microscopy. As a national user facility, the EMSL is open to the fungal biology community through a competitive, peer-reviewed proposal process.

Development and utilization of arrayed mutant sets for yeasts and filamentous fungi. Aric E Wiest, Kevin McCluskey. Fungal Genetics Stock Center, Kansas City, MO.

Advancements in high throughput functional genomics has allowed the generation of vastly increasing numbers of strains carrying single gene deletions. For some organisms these include mutations distributed across the genome. The FGSC has generated or acquired sets of arrayed mutants for several different yeast or filamentous fungal species including *Neurospora crassa, Magnaporthe grisea, Cryptococcus neoformans, Candida albicans, Aspergilus nidulans,* and *Pichia pastoris*. These arrayed sets allow rapid screening for desired traits across a broad number of gene deletions. Details of construction, replication and manipulation of these arrayed sets will be presented. Custom arraying, construction of functional sets, and cryopreservation will also be discussed.

Sequencing-based solutions to identify and characterize fungal developmental genes. Minou Nowrousian, Ines Teichert, Gabriele Wolff, Ulrich Kück. Dept. of General & Molecular Botany, Ruhr University Bochum, Bochum, Germany.

During sexual development, filamentous ascomycetes form complex, three-dimensional fruiting bodies for the protection and dispersal of spores. We are using a combination of classical genetics, next generation sequencing, molecular and microscopic methods to learn more about this differentiation process in the model organism *Sordaria macrospora*, and here we present data on the identification/characterization of transcription factors and signaling molecules that are involved in development. Whole genome sequencing of mutant pro44 was used to identify the mutation that causes sterility in the mutant strain. For Illumina/Solexa sequencing, pooled DNA from progeny of crosses of the mutant with the wild type was used, and we were able to pinpoint the causative mutation in the mutant strain through bioinformatics analysis. pro44 carries a mutation in a GATA-type transcription factor, and fertility can be restored by transformation with the wild-type allele. In a second approach, we used laser microdissection to isolate young fruiting bodies (protoperithecia) of the wild type and mutant pro1 that carries a deletion of another transcription factor gene essential for sexual development. Linear amplification of RNA from microdissected protoperithecia yielded enough material for RNA-seq analysis. A comparison with total mycelium revealed significant differences in in gene expression between protoperithecia and non-reproductive mycelia. Among the genes strongly up-regulated in protoperithecia were the pheromone precursor genes *ppg1* and *ppg2*. This was confirmed by fluorescence microscopy of *egfp* expression under control of *ppg1* regulatory sequences. In protoperithecia, many genes are under control of the transcription factor PRO1; thus, by combining laser microdissection and RNA-seq, we can now perform genome-wide analyses of genes that are dependent on a development-specific transcription factor for correct expression in a defined developmental structure in fungi. Among the genes that are dependent on PRO1 for correct expression in protoperithecia is *pro44*, which is among the 500 most strongly expressed genes in wild-type, but not pro1 protoperitheica. In summary, our data indicate that PRO1 and PRO44 are members of a transcription factor network that regulates gene expression and cell differentiation in developing fruiting bodies.

***Aspergillus nidulans* as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug resistance mutations.** Xiaoxiao Sean He, Shengnan Jill Li, Susan Kaminskyj. Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Systemic fungal infections are estimated to contribute to ~10% of hospital deaths. Systemic fungal infections are most dangerous for the young, the old, and the already sick, since their immune systems are less vigorous. Most antifungal drugs in current clinical use target ergosterol (polyenes) or the ergosterol biosynthetic pathway (azoles and allylamines). Drugs against beta-glucan synthesis (echinocandins) are effective against aspergillosis and candidaisis. The use of compounds that target fungal enzymes inevitably leads to the development and natural selection of drug resistant fungal strains. Not only are the anti-fungal drugs in current clinical use losing efficacy in some situations, but in addition the high level of conservation between animal and fungal physiology leaves relatively few relevant targets to explore. However, it is likely that for any drug-enzyme combination there will be relatively few mutations that could increase drug resistance while still maintaining enzyme function. We are using *Aspergillus nidulans* as an experimental model system to assess the number and identity of mutations that lead to drug resistance. As proof of concept, we grew wild type *A. nidulans* on replicate plates containing a sub-lethal concentration of Calcofluor. These developed fast-growing sectors beginning at ~ 5 d (70 rounds of mitosis). Preliminary results show that many of these sectors harboured heritable, single-gene mutations. To date, mutated genes that confer robust, heritable resistance to Calcofluor that were identified by next generation sequencing have roles in cell wall synthesis, cell wall integrity regulation, or drug detoxification. We suggest this strategy will be useful for predicting genetically-mediated anti fungal resistance adaptation and help us to be ahead in the drug-resistance arms race.

Illumina-based genetic linkage map for wheat leaf rust. David L. Joly[1,2], Barbara Mulock[3], Christina A. Cuomo[4], Barry J. Saville[2], Brent D. McCallum[3], Guus Bakkeren[2]. 1) Pacific Agri-Food Research Centre, Agriculutre and Agri-Food Canada, Summerland, British Columbia, Canada; 2) Forensic Science Program and Environmental & Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada; 3) Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada; 4) Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA 02142.

Few genetic maps have been made for rust fungi; yet they are useful in identifying candidate loci for phenotypic traits or in unravelling chromosomal arrangements. This lack of maps is, in part, due to the obligate biotrophic nature of rusts and the difficulties in manipulating their life cycle in a way that enables controlled crosses. Recently, the genome sequence of a wheat leaf rust (Puccinia triticina) isolate was determined and this prompted the sequencing of additional isolates using next-generation sequencing technologies. This has dramatically increased the amount of sequence information available at a substantially decreased per base cost. Fifty-seven F2 progeny of a wheat leaf rust sexual cross between race 9 (SBDG) and race 161 (FBDJ) were sequenced using Illumina. In order to generate a high-resolution genetic linkage map, genome-wide single-nucleotide polymorphisms (SNPs) were identified. Employing the genome sequence information from the two parents and the F1 isolate, more than 25,000 SNPs were selected and used to generate a genetic linkage map. Although they were obtained from different isolates, the genetic map and the reference genome were integrated, allowing the creation of pseudomolecules. Those represent a strong improvement over the currently fragmented status of the reference genome. Moreover, at least 9 seedling and 2 adult-plant avirulence genes were shown to segregate in this F2 population and candidate genes identified using the genetic map are currently being investigated.

Peering into the secret-ory life of *Aspergillus nidulans* with a little help from classical genetics. Miguel Penalva[1], Areti Pantazopoulou[1], Mario Pinar[1], Herbert N. Arst, Jr.[2]. 1) Cellular and Molecular Biology, Centro de Investigaciones Biologicas CSIC, Madrid, Spain; 2) Department of Microbiology, Imperial College, London, UK.

Model fungi have survived the revolution of modern biology partly through their amenability to classical genetic analysis. Unquestionably, classical genetics lay at the root of the unmatched success of the yeast *Saccharomyces cerevisiae*, that exotic fungal visitor so pleasantly accepted into the parlour of true eukaryotic cells and in the conservatory of gene regulation that dominated the fungal community at the end of last millennium. Formerly fashionable, classical genetics became nearly extinct with the advent of the '*omics* era', their demise confirmed with each of the uncountable developments of low-cost sequencing. However, we shall illustrate how extraordinarily powerful classical genetics can be, used in combination with sequencing techniques, to address general questions on the organization of the Golgi in eukaryotic cells. The Golgi is essential for secretion, and therefore, for hyphal growth. Thus, we begin with a sequenced, well-characterized heat-sensitive X^{ts} mutation in an *A. nidulans* Golgi gene. An X^{ts} strain is mutagenised to isolate *suX* suppressor mutations, reversing the absence of growth resulting from X^{ts} at the restrictive temperature. Less interesting intragenic reversion/pseudo-reversion events are identified by the inability of any given *suX* X^{ts} strain to produce single mutant X^{ts} progeny when crossed to a wild-type. These mutations are next sequenced and archived. The remaining extragenic suppressors are allocated to one of the eight *A. nidulans* chromosomes by parasexual analysis, exploiting the rarity of mitotic recombination. Next, meiotic crosses between the *suX* X^{ts} strain and a panel of parental strains carrying markers in the *suX* chromosome are analysed to detect genetic linkage. Once linkage is detected, *suX* is further mapped to the smallest feasible chromosomal interval. Candidate genes in the annotated genome interval, hopefully conspicuous at this stage to the educated eye, or, as a last resort, the whole interval between the genetic boundaries, are sequenced to identify the suppressor. The combination of gene mapping with sequencing eliminates the cumbersome identification of a single causative mutation (aka 'a needle in a haystack') hidden amongst the genetic variability of the mutant and parental strains, inherent to whole genome sequencing approaches.

Domains of meiotic DNA recombination and gene conversion in *Coprinopsis cinerea* (*Coprinus cinereus*). Patricia J. Pukkila[1], Wendy Schackwitz[2]. 1) Dept Biol, Univ North Carolina, Chapel Hill, NC, USA; 2) US DOE Joint Genome Institute, Walnut Creek, CA, USA.

We have shown previously that rates of meiotic recombination are highly non-uniform along the assembled chromosomes of *C. cinerea* (Stajich et al. PNAS 107: 11889-11894, 2010). That study revealed an over-representation of paralogous multicopy genes in regions with elevated levels of meiotic exchange. In addition, retrotransposon-related sequences were not found in large segments of the genome with low levels of meiotic exchange. However, the study was limited by the available markers, and only 31 Mb of the 36 Mb genome could be mapped. More recently, we have resequenced 45 meiotic segregants and 4 complete tetrads. We developed a simple script to detect crossover and gene conversion events involving over 75,000 SNPs spanning 35 Mb. The data were analyzed using MSTmap (Wu et al. PLoS Genetics 4: e1000212, 2008). The new dataset revealed sub-telomeric recombination hotspots at every chromosome end, and 36% of the crossovers were associated with uninterrupted tracts of gene conversion. The conversion tracts (2-8 SNPs) were quite short (8-219 nt), and the median distance between the flanking SNP markers was also small (500 nt). Since these subtelomeric hotspots correspond to sites of synaptic initiation in *C. cinerea* (Holm et al. Carlberg Res. Commun. 46: 305-346, 1981), these data may contribute to our understanding of how homologous chromosome pairing and synapsis are coordinated with meiotic recombination. Supported by the U.S. Department of Energy Joint Genome Institute Community Sequencing Program. The work conducted by the U.S. DOE JGI is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

A Hook protein is critical for dynein-mediated early endosome movement in Aspergillus nidulans. Jun Zhang[1], Rongde Qiu[1], Herbert Arst[2], Miguel Peñalva[3], Xin Xiang[1]. 1) Department of Biochemistry and Molecular Biology, Uniformed Services University, Bethesda, Maryland, USA; 2) Department of Medicine, Imperial College London, London, UK; 3) Department of Molecular and Cellular Medicine, Centro de Investigaciones Biológicas CSIC, Ramiro de Maeztu 9, Madrid, Spain.

It has been hypothesized that cytoplasmic linker proteins such as CLIP-170 facilitate motor-driven organelle transport by serving as an additional linker between the organelle and the microtubule track. However, mammalian and fungal cells lacking CLIP-170 do not exhibit any apparent defects in vesicle transport. We recently found that in the filamentous fungus Aspergillus nidulans, the HooK protein ortholog, HookA, is critical for dynein-mediated transport of early endosomes. HookA mutants were obtained from a genetic screen for mutants defective in dynein-mediated early endosome movement, and the HookA gene was identified by a combination of classical genetic and whole-genome-sequencing approaches. The HookA protein is homologous to human Hook proteins containing a N-terminal microtubule-binding domain, a coiled-coil domain and a C-terminal cargo-binding domain, an organization similar to that of CLIP-170. Both the N- and C-terminal domains of HookA are required for dynein-mediated early endosome transport, and HookA associates with early endosomes via its C-terminal domain in a dynein-independent manner. Importantly, HookA physically interacts with dynein/dynactin, and this interaction is independent of the C-terminal early-endosome-binding domain but dependent upon the N-terminal microtubule-binding domain. Together, our results suggest that HookA may facilitate cargo-motor-track interactions during dynein-mediated transport of early endosomes.

Thursday, March 14 3:00 PM–6:00 PM
Chapel

Fungi and Evolutionary Theory

Co-chairs: Hanna Johannesson and Duur Aanen

Reaching the wind: the fluid mechanics of spore discharge, and potential for dispersal mechanisms to shape the evolution of sporocarp and spore morphologies. Anne Pringle[1], Michael Brenner[2], Joerg Fritz[2], Marcus Roper[3], Agnese Seminara[2]. 1) Organismic & Evolutionary Biology, Harvard University, Cambridge, MA; 2) School of Engineering and Applied Sciences, Harvard University, Cambridge, MA; 3) Department of Mathematics, University of California, Los Angeles, CA.

Fungi play critical roles in human agriculture and Earth's biogeochemistry, but mechanisms of fungal dispersal are poorly understood. Thinking has focused on the passive spread of spores by air and water, and neglected the biomechanics used by fungi to actively move spores to new habitats. In this talk we focus on terrestrial ascomycetes, a group including plant and animal pathogens, mycorrhizal fungi, and lichens. We build theory to catalog and explain the morphological features used by ascomycetes to shoot spores and facilitate the crossing of the boundary layer, a sheath of nearly still air surrounding sporocarps. Crossing the boundary layer is critically important to the fitness of a spore: a spore that cannot escape will fall back on the parent fungus, where probabilities of germination and survival are low. But after crossing the boundary layer, a spore must also travel in wind, and by explicitly modeling discharge and dispersal we identify a previously unsuspected trade-off constraining the sizes of spores. Large spores cross boundary layers more effectively, while small spores are more easily carried by wind. Spore dispersal shapes the epidemiology of disease, and will mediate range shifts in response to global change; understanding how and how quickly fungi move across landscapes will enable both management and conservation.

***Neurospora tetrasperma* mating-type chromosomes: Testing hypotheses on the effects of degeneration and introgression on performance.** Jennifer L. Anderson, Yu Sun, Pádraic Corcoran, Hanna Johannesson. Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden.

Following hybridization between species, parts of one species' genome can be incorporated into the genome of the other. This transfer of genetic material, introgressive hybridization, is a well-known driver of speciation, diversification, and adaptive evolution. Introgression has occurred repeatedly in the fungus *Neurospora tetrasperma* and has resulted in the presence of large regions of DNA (< 4 Mbp tracts) from other species of *Neurospora* on the mating-type (*mat*) chromosomes of *N. tetrasperma*. The *mat* chromosomes of *N. tetrasperma* also contain large regions of suppressed recombination that are associated with the accumulation of mutations and possibly a reduction in biological fitness. It has been proposed that introgressions of DNA from other taxa, with freely recombining *mat* chromosomes, onto the *mat* chromosomes of *N. tetrasperma* could counteract the deleterious effects of mutation accumulation and "reinvigorate" fitness. Alternatively, interspecific introgression into *N. tetrasperma mat* chromosomes could be either neutral or deleterious to fitness, but are maintained due to lack of recombination between *mat* chromosomes. To test these hypotheses we have quantified physiological performance (linear growth rate, LGR) in homokaryons from eight strains of *N. tetrasperma* with *mat* chromosomes that differ in introgression history (*e.g.* introgressions from different species) and degree of degeneration. Differences in LGR between mating types and chromosome types (introgressed or degenerate) will inform our understanding how hybridization and chromosomal structure and content effect physiological performance and possibly fitness.

Nuclear arms races: sexual selection for masculine mushrooms. Bart Nieuwenhuis, Duur Aanen. Laboratory of Genetics, Wageningen University, Wageningen, Netherlands.

When many gametes compete to fertilize a limited number of compatible gametes, sexual selection will favor those traits that increase competitive advantage during mating. In animals and plants, sperm and pollen competition have yielded many interesting adaptations for improved mating success. In fungi, similar processes have not been directly shown yet. We test the hypothesis that sexual selection can increase competitive fitness during mating, using experimental evolution in the mushroom fungus *Schizophyllum commune*. Mating in *S. commune* occurs by donation of nuclei to a mycelium. These fertilizing 'male' nuclei migrate through the receiving 'female' mycelium. In our setup, an evolving population of nuclei was serially mated with a non-evolving female mycelium for 20 sexual generations. Four of the 12 tested strains had significantly increased competitive fitness and one had decreased fitness. The main characteristic that explained fitness change was the relative success in colonization of the female mycelium. In most cases, no trade-offs were found with other fitness components. Our results show that sexual selection can act in mushroom fungi and that sexual selection can lead to increased competitive ability during mating.

Genome-wide mutation dynamic within a long-lived individual of Armillaria. James B. Anderson. Deptartment of Biology,, Univ Toronto, Mississauga, Ontario, Canada.

Mutation is the ultimate source of all genetic variation in populations and yet the events themselves remain unobservable and buried in the past. Long-lived individuals of *Armillaria gallica*, a common opportunistic fungal pathogen of tree roots in temperate forests of the northern hemisphere, provide a spatial context for the mutational dynamic. Each individual of *A. gallica* arises in a single mating event between two haploid gametes and the resulting diploid genotype then grows vegetatively to occupy a discrete spatial territory including many adjacent tree root systems. In effect, this leaves a spatial record of growth over time within which mutations can be pinpointed. To identify mutations, the entire genomes of three spatially separated samples of one individual of *A. gallica* approximately 200 by 60 m in size were sequenced and compared. In this comparison, mutations and chromosomal regions of loss of heterozygosity (LOH) were identified and then assayed in another 22 isolates from the same individual.by conventional PCR and Sanger sequencing. The genotype network of all 10 mutations and two LOH events in the 90 MB genome assembly was without internal conflict. Further, the spatial distribution of genotypes was non-random and appeared to reflect the vegetative expansion leading to the present-day individual. I will discuss the implications of the whole-genome data in estimating mutation rates and cellular generation times.

Rapid genetic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. C. Angelard, I. Sanders. University of Lausanne, Biophore, 1015 Lausanne, Switzerland.

Arbuscular mycorrhizal fungi (AMF) are among the most abundant symbionts of plants, improving plant productivity and diversity. They are clonal; a trait assumed to limit adaptability. However, AMF harbour genetically different nuclei. We hypothesized that AMF can respond rapidly to a change of environment through changes in the frequency of nuclei and by making genetically novel offspring. We subjected AMF parents and offspring to a host shift. We observed genetic changes in all AMF lines. Genetic and phenotypic responses were different among offspring and some displayed higher fitness than their parents. Our results demonstrate that AMF rapidly undergo genetic change in response to the environment and that nucleotype frequency plays a role in how they perform in the new environment. Even though clonal, AMF offspring display greater genetic change and plasticity in response to host shift. Such genetic and phenotypic flexibility is likely to be key to their ecological success.

Meiotic Drive: A Single Gene Conferring Killing and Resistance in Fungal Spore Killer. Pierre Grognet[1,2*], Fabienne Malagnac[1,2], Hervé Lalucque[1,2], Philippe Silar[1,2]. 1) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain, 75205 Paris CEDEX 13 France; 2) Univ Paris Sud, Institut de Génétique et Microbiologie, Bât. 400, 91405 Orsay cedex, France.

Meiotic drives (MD) are nuclear genetic loci ubiquitous in eukaryotic genomes that cheat the Mendel laws by distorting segregation in their favor. All known MD are composed of at least two linked genes, the distorter that acts as a toxin by disrupting the formation of gametes, and the responder that acts as an antitoxin and protects from the deleterious distorter effects. In fungi, MDs are known as Spore Killers (SK). In the model ascomycete *Podospora anserina*, MD has been associated with deleterious effect during ascospore formation of the Het-s prion and in *Neurospora crassa* a resistance gene (responder) to the Sk-2 and Sk-3 distorters has been identified. MDs are easily studied in *P. anserina* thanks to the ascus structure as SKs are identified by the presence of 2-spored asci in crosses between strains. Here, we identify and characterize by targeted deletion in *P. anserina Spok1* and *Spok2*, two MD elements. We show that they are related genes with both spore-killing distorter and spore-protecting responder activities carried out by the same allele, unlike other known MD. These alleles act as autonomous elements and exert their effects in any region of the genome. Moreover, *Spok1* acts as a resistance factor to *Spok2* killing. As *Spok1* and *Spok2* belong to a multigene family, these Spore Killer genes represent a novel kind of selfish genes that proliferate in population through meiotic distortion.

Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta. Pierre Gladieux, David Kowbel, Christopher Hann-Soden, John Taylor. Department of Plant and Microbial Biology, University of California, Berkeley, CA.

Identifying the genes for ecologically relevant traits is a central challenge in empirical population genetics. Species distributed across strong environmental gradients are excellent models to discover and identify the genetic targets of local selection as they are more likely to experience spatially heterogeneous selection pressures leading to local adaptation of ecologically important traits. We studied the origin of ecological differentiation in N. discreta phylogenetic species 4 (PS4), a species with a broad latitudinal distribution. We Illumina-sequenced the complete genomes of 52 individuals representing 8 collections sites in Alaska, New Mexico, Washington, California, and Western Europe (average sequencing depth: 52X). Reads were mapped to the N. discreta PS4 reference genomes, and analyses were based on a final set of ca. 1.2 million high-quality SNPs. Phylogenetic analyses identified four well-supported clades. Papua New-Guinea individuals formed the most basal clade. Individuals from Alaska and Europe on the one hand, and from New Mexico on the other hand grouped into sister clades, and individuals from California were basal to these two clades. Individuals from Washington, sampled within the same site, grouped with either the New Mexico individuals, or the California individuals, indicating the coexistence in sympatry of two divergent populations. The observed pattern of population subdivision is being used as a reference to identify genes departing from the genome-wide background, and showing increased divergence consistent with divergent selective pressures, or decreased divergence consistent with gene-flow. Our findings emphasize the need to continue exploration to uncover divergent populations of Neurospora, and place N. discreta, along with N. crassa, among the handful of species that have the attributes to serve as outstanding evolutionary and ecological model organisms.

Ecological context in symbioses: when is your enemy also your friend? Georgiana May[1], Paul Nelson[2]. 1) Dept Ecol, Evol, Behavior,#100, Univ Minnesota, St Paul, MN; 2) EEB graduate program University of Minnesota St. Paul MN.

Most plants are rife with fungal symbiotic partners with many of these having little apparent effect on the host's health and fitness. In this work, we explore the degree to which the outcome of interactions between an endophytic fungus, pathogen and plant host depend on ecological context. In particular, we ask whether interactions between the endophyte of maize, Fusarium verticilliodes, with the pathogen Ustilago maydis, depend on host resistance to the pathogen. In the case of a host susceptible to the pathogen, the two fungal species should meet frequently, and compete over host resources, potentially driving greater virulence to the host in one or the other fungal species. In the case of a host resistant to the pathogen, the endophyte might be a "bystander" to the pathogen, because the two meet too infrequently to drive their co-evolutionary interaction. We show evidence that the two fungal species have evolved stronger antagonistic interactions in maize susceptible to the pathogen, and further, that this might be associated with greater virulence by the pathogen. Results of modeling will also be presented from which we predict longer term evolutionary trajectories for this 3-way interaction.

Thursday, March 14 3:00 PM–6:00 PM
Heather

Cytoskeleton, Motors, and Intracellular Transport

Co-chairs: Samara Reck-Peterson and Ping Wang

The molecular basis of extended dynein run-length. Sreedhar Kilaru, Martin Schuster, Gero Steinberg. School of Bioscinces, Univ Exeter, EX4 4QD Exeter, UK.

Dynein is a minus-end directed motor that utilises ATP to transport organelles along microtubules. In fungi, a major "cargo" of dynein are early endosomes that are taken over long distance from the plus-ends near the growing apex to the central part of the hyphal cell. In cell-free assays it was shown that single dynein motors can only overcome 1 micrometer, and long-distance motility of organelles requires binding of several dynein motors that cooperate to extend the transport distance. We recently showed that this does not apply to the fungus *Ustilago maydis*. Here, single dynein motors move over 30 micrometers, raising the question of the underlying molecular mechanism for this extraordinary motor performance. This talk will provide a comprehensive explanation for this phenomenon.

The role of microtubule-based motors in the spatiotemporal control of autophagy. Martin Egan, Mark McClintock, Samara Reck-Peterson. Cell Biology, Harvard Medical School, Boston, MA.

Autophagy is a highly conserved eukaryotic process in which components of the cytoplasm, including damaged organelles and misfolded proteins, are sequestered into double membrane-bound vesicles called autophagosomes that are subsequently delivered to the vacuole for recycling. In fungi, autophagy is linked to cellular remodeling and differentiation, while in mammals dysfunction in the autophagy pathway has been implicated in cancer and neurodegenerative diseases. Here we explore the role of microtubule-based motors in the spatiotemporal control of autophagy in the model filamentous fungus Aspergillus nidulans. Using a molecular genetic and live-cell imaging approach, we identify the motors responsible for autophagosome motility, and dissect their role in the delivery and fusion of autophagic vesicles with the vacuolar system. Furthermore, we examine the role of microtubule-based motors in the clearance of aggregation-prone proteins associated with motor neuron disease, and determine the effect of these aggregates on normal microtubule-based transport processes.

Microtubule-dependent co-transport of mRNPs and endosomes. Sebastian Baumann[1,2], Thomas Pohlmann[1,2], Andreas Brachmann[2,3], Michael Feldbrügge[1,2]. 1) Heinrich-Heine University Düsseldorf, Institute for Microbiology, 40204 Düsseldorf, Germany; 2) Max Planck Institute for Terrestrial Microbiolgy, Department of Organismic Interactions, Karl-von-Frisch-Str. 10, 45043 Marburg, Germany; 3) Biocenter of the Ludwigs Maximilians University Munich, Genetics Section, Grosshaderner Str. 2-4, 82152 Planegg-Martinsried, Germany.

Long-distance transport of mRNAs is important in determining polarity in eukaryotes. Molecular motors shuttle large messenger ribonucleoprotein complexes (mRNPs) containing mRNAs, RNA-binding proteins and associated factors along microtubules. However, precise mechanisms including the interplay of molecular motors and a potential connection to membrane trafficking remain elusive. In recent studies we identified the RNA-binding protein Rrm4 as the key player in microtubule-dependent mRNA transport in *Ustilago maydis*. Combining *in vivo* CLIP and RNA-live imaging revealed a subset of mRNAs that are bound by Rrm4 and transported processively throughout the hyphae. Studying the molecular motors revealed that shuttling is mediated by Kin3 and Dyn1/2. The same set of motors acts in endosome trafficking and indeed, studying the SNARE Yup1 and the small GTPase Rab5 we found co-transport with endosomes as a novel mechanism for mRNP transport. Currently, we address the link between mRNAs and endosomes.

Role of *tea1* and *tea4* homologs in cell morphogenesis in *Ustilago maydis*. Flora Banuett, Woraratanadharm Tad, Lu Ching-yu, Valinluck Michael. Biological Sciences, California State University, Long Beach, CA.

We are interested in understanding the molecular mechanisms that govern cell morphogenesis in *Ustilago maydis*. This fungus is a member of the Basidiomycota and exhibits a yeast-like and a filamentous form. The latter induces tumor formation in maize (*Zea mays*) and teosinte (*Zea mays* subsp. *parviglumis* and subsp. *mexicana*). We used a genetic screen to isolate mutants with altered cell morphology and defects in nuclear position. One of the mutants led to identification of *tea4*. Tea4 was first identified in *Schizosaccharomyces pombe*, where it interacts with Tea1 and other proteins that determine the axis of polarized growth. Tea4 recruits a formin (For3), which nucleates actin cables towards the site of growth, and thus, polarizes secretion (Martin et al., 2005). Tea1 and Tea4 have been characterized in *Aspergillus nidulans* and *Magnaporthe oryzae* (Higashitsuji et al., 2009; Patkar et al., 2010; Takeshita et al., 2008; Yasin et al., 2012). Here we report the characterization for the first time of the Tea4 and Tea1 homologs in the Basidiomycota. The *U. maydis tea4* ORF has coding information for a protein of 1684 amino acid residues that contains a Src homology (SH3) domain, a RAS-associating domain, a phosphatase binding domain, a putative NLS, and a conserved domain of unknown function. All Tea4 homologs in the Basidiomycota contain a RA domain. This domain is absent in Tea4 homologs in the Ascomycota, suggesting that Tea4 performs additional functions in the Basidiomycota. We also identified the Um*tea1* homolog, which codes for a putative protein of 1698 amino acid residues. It contains three Kelch repeats. The Tea1 homologs in the Ascomycota and Basidiomycota contain variable numbers of Kelch repeats. The Kelch repeat is a protein domain involved in protein-protein interactions. The *tea1* gene was first identified in *S. pombe* and is a key determinant of directionality of polarized growth (Mata and Nurse, 1997). To understand the function of *tea1* and *tea4* in several cellular processes in *U. maydis*, we generated null mutations. We demonstrate that *tea4* and *tea1* are necessary for the axis of polarized growth, cell polarity, normal septum positioning, and organization of the microbutubule cytoskeleton. We also determined the subcellular localization of Tea1::GFP and Tea4::GFP in the yeast-like and filamentous forms.

Aspergillus nidulans **septin interactions and post-translational modifications.** Yainitza Hernandez-Rodriguez[1], Shunsuke Masuo[2], Darryl Johnson[3], Ron Orlando[3,4], Michelle Momany[1]. 1) Plant Biology, University of Georgia, Athens, GA, US; 2) Laboratory of Advanced Research A515, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, JP; 3) Department of Chemistry, University of Georgia, Athens, GA, US; 4) Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, US.

Septins are cytoskeletal elements found in fungi, animals, and some algae, but absent in higher plants. These evolutionarily conserved GTP binding proteins form heteroligomeric complexes that seem to be key for the diverse cellular functions and processes that septins carry out. Here we used *Aspergillus nidulans*, a model filamentous fungus with well defined vegetative growth stages to investigate septin-septin interactions. *A. nidulans* has five septins: AspA/Cdc11, AspB/Cdc3, AspC/Cdc12 and AspD/Cdc10 are orthologs of the "core-filament forming-septins" in *S. cerevisiae*; while AspE is only found in filamentous fungi. Using S-tag affinity purification assays and mass spectrometry we found that AspA, AspB, AspC and AspD strongly interact in early unicellular and multicellular vegetative growth. In contrast, AspE appeared to have little or no interactions with core septins in unicellular stages before septation. However, after septation AspE interacted with other septins, for which we postulate an accessory role. AspE localized to the cortex of actively growing areas and to septa, and localizations are dependent on other septin partners. Interestingly, core septin localizations can also depend on accessory septin AspE, particularly post-septation. In addition, LC-MS/MS showed acetylation of lysine residues in AspA before septation and AspC after septation. Western blot analysis using an anti-acetylated lysine antibody showed that AspC is highly acetylated in all stages examined, while other septins showed acetylation post-septation. Though LC-MS analysis failed to detect phosphorylation of septins, this modification has been widely reported in fungal septins. Using phosphatase treatments and Western Bloting, we found phosphorylation of AspD, but no other septins. This is interesting because AspD belongs to a special group of septins that lack a C-terminal coiled-coil found in other septins. However, septin localization is not affected by the absence of AspD/Cdc10, but by the absence of filamentous fungi specific septin AspE. Our data suggests that septin interactions and modifications change during development and growth in *A. nidulans*, and that some modifications are septin specific.

Altered Ras1 trafficking impairs the pathogencity of ***Cryptococcus neoformans*****.** Connie B. Nichols, Teresa O'Meara, Kyla Selvig, Sandra Breeding, J. Andrew Alspaugh. Dept. of Medicine, Duke University Medical Center, Durham, NC, USA.

Cryptococcus neoformans is an opportunistic human fungal pathogen. The ability to cause disease is linked to several different determinants, one of which is the ability to grow at high temperature. Previously we found that one branch of the Ras1 signaling cascade mediates cell morphology and cytokinesis in response to mild stress, such as growth at high temperature. Inactivation of Ras1 and other components of this signaling branch negatively impacts *C. neoformans* pathogenicity. Additionally, this branch of the Ras1 signaling cascade is dependent on the trafficking of Ras1 from the endomembranes to the plasma membrane and is mediated by palmitoylation of the Ras1 protein. We have identified and characterized several *C. neoformans* protein acyltransferases (PATs), the enzymes responsible for palmitoylation, to further understand the role of palmitoylation and trafficking on Ras1 function and activity during high temperature growth and pathogenesis. Although there is some degree of functional redundancy in this protein family, we identified individual PATs that are required for stress response and virulence in models of cryptococcal disease.

Quantification of the thigmotropic response of ***Neurospora crassa*** **to microfabricated slides with ridges of defined height and topography.** Karen Stephenson[1], Fordyce Davidson[2], Neil Gow[3], Geoffrey Gadd[1]. 1) Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, United Kingdom; 2) Division of Mathematics, University of Dundee, Dundee, United Kingdom; 3) Institute of Medical Sciences, University of Aberdeen, Aberdee, United Kingdom.

Thigmotropism is the ability of an organism to exhibit an orientation response to a mechanical stimulus. We have quantified the thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography. We show that mutants that lack the formin BNI-1 and the Rho-GTPase CDC-42, an activator of BNI-1, had an attenuated thigmotropic response. In contrast, null mutants that lacked cell end-marker protein TEA-1 and KIP-A, the kinesin responsible for its localisation, exhibited significantly increased thigmotropism. These results indicate that vesicle delivery to the hyphal tip via the actin cytoskeleton is critical for thigmotropism. Disruption of actin in the region of the hyphal tip which contacts obstacles such as ridges on microfabricated slides may lead to a bias in vesicle delivery to one area of the tip and therefore a change in hyphal growth orientation. This mechanism may differ to that reported in *Candida albicans* in so far as it does not seem to be dependent on the mechanosensitive calcium channel protein Mid1. The *N. crassa* D*mid-1* mutant was not affected in its thigmotropic response. Although it was found that depletion of exogenous calcium did not affect the thigmotropic response, deletion of the *spray* gene, which encodes an intracellular calcium channel with a role in maintenance of the tip-high calcium gradient, resulted in a decrease in the thigmotropic response of *N. crassa*. This predicts a role for calcium in the thigmotropic response. Our findings suggest that thigmotropism in *C. albicans* and *N. crassa* are similar in being dependent on the regulation of the vectorial supply of secretory vesicles, but different in the extent to which this process is dependent on local calcium-ion gradients.

Dynein drives oscillatory nuclear movements in the phytopathogenic fungus ***Ashbya gossypii*** **and prevents nuclear clustering.** S. Grava, M. Keller, S. Voegeli, S. Seger, C. Lang, P. Philippsen. Biozentrum, Molecular Microbiology, University of Basel, CH 4056 Basel, Switzerland.

In the yeast Saccharomyces cerevisiae the dynein pathway has a specific cellular function. It acts together with the Kar9 pathway to position the nucleus at the bud neck and to direct the pulling of one daughter nucleus into the bud. Nuclei in the closely related multinucleated filamentous fungus Ashbya gossypii are in continuous motion and nuclear positioning or spindle orientation is not an issue. A. gossypii expresses homologues of all components of the Kar9/Dyn1 pathway, which apparently have adapted novel functions. Previous studies with A. gossypii revealed autonomous nuclear divisions and, emanating from each MTOC, an autonomous cytoplasmic microtubule (cMT) cytoskeleton responsible for pulling of nuclei in both directions of the hyphal growth axis. We now show that dynein is the sole motor for bidirectional movements. Surprisingly, deletion of Kar9 shows no phenotype. Dyn1, the dynactin component Jnm1, the accessory proteins Dyn2 and Ndl1, and the potential dynein cortical anchor Num1 are involved in the dynamic distribution of nuclei. In their absence, nuclei aggregate to different degrees, whereby the mutants with dense nuclear clusters grow extremely long cMTs. Like in budding yeast, we found that dynein is delivered to cMT +ends, and its activity or processivity is probably controlled by dynactin and Num1. Together with its role in powering nuclear movements, we propose that dynein also plays (directly or indirectly) a role in the control of cMT length. Those combined dynein actions prevent nuclear clustering in A. gossypii and thus reveal a novel cellular role for dynein.

Thursday, March 14 3:00 PM–6:00 PM
Fred Farr Forum

Nucleic Acid-Protein Interactions that Impact Transcription and Translation
Co-chairs: Michael Freitag and Mark Caddick

ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi. Koon Ho Wong, Kevin Struhl. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

Chromatin Immuno-precipitation (ChIP) is a commonly used technique for studying protein-DNA interactions. When coupled with the Next Generation Sequencing (NGS) technology, ChIP-seq can map and measure genome-wide locations and occupancies of any protein-of-interest at very high resolution, and is an invaluable technique for studying chromatin-associated processes including transcription regulation. However, owing to the fact that NGS experiments are expensive, this powerful technique has yet been widely applied to fungal studies. The output of current sequencing technologies vastly exceeds the sequencing depth requirement of ChIP-seq experiments as well as many NGS applications in fungi. We have developed a multiplex sequencing method that allows up to 96 different samples to be included in a single sequencing reaction, providing a means to obtain whole-genome data at a highly affordable cost. Using multiplex sequencing, ChIP-seq and a technique called Anchor-Away for conditional depletion of proteins from the nucleus, we have gained important insights into different aspects of transcription regulation including the repression mechanism of the Cyc8-Tup1 co-repressor complex in *Saccharomyces cerevisiae*. Examples on how ChIP-seq applications may be broadly applied to address common questions regarding transcription regulation will also be presented.

Regulatory Networks Governing Global Responses to Changes in Light and Time. Jay C. Dunlap, Jennifer J. Loros, & the P01 Consortium**"Functional Analysis and Systems Biology of Model Filamentous Fungi". coordinated from Dept Gen, Geisel School of Medicine at Dartmouth, Hanover, NH.

**including PIs Deb Bell-Pedersen, Michael Freitag, James Galagan, Matthew Sachs, Eric Selker, Jeff Townsend, and members of their labs at institutions not listed here. Free-living fungi live in a profoundly rhythmic environment characterized by daily changes in light intensity and temperature. Some fungi have well described systems for anticipation of temporal change, circadian systems, and nearly all fungi can respond acutely to changes in light intensity. The nuts and bolts of the regulatory structures underlying circadian regulation and responses to blue light are well known in Neurospora. The circadian clock comprises a negative feedback loop wherein a heterodimer of proteins, WC-1 and WC-2, acts as a transcription factor (TF) to drive expression of frq. FRQ stably interacts with a putative RNA helicase (FRH) and with casein kinase 1, and the complex down-regulates the White Collar Complex (WCC). With appropriate phosphorylation mediated delays, this feedback loop oscillates once per day (Baker, Loros, & Dunlap, FEMS Microbiol. Reviews 36: 95-106, 2012). In turn, blue light is detected by FAD stably bound by WC-1, eliciting photochemistry that drives a conformational change in the WCC resulting in activation of gene expression from promoters bound by the WCC (Chen, Dunlap & Loros, FGB 47, 922-9, 2010). With this as context, the consortium team listed above is using the tools of next generation sequencing, recombineering and luciferase reporters to see how the initial simple steps of clock control and light perception ramify via regulatory networks to elicit development in response to the cues of light and time. Interestingly, the same players and networks appear to be involved in many places. For instance, the circadian feedback loop yields rhythmic activation of WCC that regulates many genes including transcription factors (TFs). Genes encoding TFs that do not affect the circadian feedback loop itself provide circadian output. In this manner these TFs act as second order regulators, transducing regulation from light responses or from the core circadian oscillator, to banks of output clock-controlled genes (ccgs), some of which are in turn other TFs. Assembling the global regulatory networks governing light and clock regulation is now a feasible goal.

Protein Binding Microarrays and *high-throughput* real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in *Neurospora crassa*. A. Montenegro-Montero[1], A. Goity[1], C. Olivares-Yañez[1], A. Stevens-Lagos[1], M. Weirauch[2], A. Yang[3], T. Hughes[3], L. F. Larrondo[1]. 1) Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) CAGE, Cincinnati Children`'s Hospital Medical Center, University of Cincinnati. U.S.A; 3) Banting and Best Department of Medical Research, University of Toronto, Canada.

It has been suggested that ~20% of the Neurospora-transcriptome may be under circadian control. Nevertheless, there is scarce information regarding the regulators that are involved in the rhythmic expression of *clock-controlled genes* (*ccg*s). We are using a *high-throughput* platform, based on various codon-optimized luciferase transcriptional- and translational-reporters, to monitor time-of-day-specific gene expression and to identify key elements mediating circadian transcriptional control. Thus, we have identified transcription factors -such as SUB-1- that affect the expression of known and novel *ccg*s, among which there are transcriptional regulators that give access to a group of third-tier *ccg*s. In addition, we are characterizing several rhythmic bZIP-coding genes as potential nodes of circadian regulation. In order to characterize regulatory networks in which these and all Neurospora transcription factors participate, we are using double-stranded DNA microarrays containing all possible 10-base pair sequences to examine their binding specificities and in that way, predict possible targets on a genome-wide manner. Currently, these Protein Binding Microarray studies have provided DNA-binding specificities for over 120 Neurospora transcription factors granting an unprecedented and powerful tool for transcriptional network studies. Finally, we have generated graphic tools to explore the spatial differences observed in the temporal control of gene expression. Funding: Conicyt/Fondecyt/regular 1090513.

Ending messages: alternative polyadenylation in filamentous fungi. Julio Rodriguez-Romero, Ane Sesma. CBGP/ Univ Politécnica de Madrid, Pozuelo de Alarcón, Madrid, Spain.

The 3' end polyadenylation of pre-mRNAs is a two-step process. First, pre-mRNAs are cleaved at their 3' end. The second step involves the addition of the polyA tail by RNA polymerases. Presence of multiple potential 3' end cleavage sites is common in eukaryotic genes, and the selection of the right site is regulated during development and in response to cellular cues. This mechanism of alternative (or non-canonical) polyadenylation generates mRNA isoforms with different exon content or 3' UTR lengths and regulates the presence of *cis* elements in the mRNA. Proteins involved in alternative polyadenylation (APA) include Cleavage Factor I in metazoans (CFIm), Hrp1 in yeast and Rbp35 in filamentous fungi. The *cis* elements present in the 3' UTRs such as miRNA target sites modulate gene expression by affecting cytoplasmic polyadenylation, subcellular localization, stability, translation and/or decay of the mRNA. Therefore, the selection of a proper 3' end cleavage site represents an important step of regulation of gene expression. Using Direct RNA Sequencing (DRS), we are carrying out in the rice blast fungus *Magnaporthe oryzae* a comprehensive map of genome wide polyadenylation sites and

quantifying their usage under different nutritional conditions (rich and minimal media, carbon and nitrogen starvation) in the wild type and the *Drbp35* mutant. Results of these polyadenylation maps will be presented, including candidate APA targets, sequence motifs present in long 3' UTRs, Rbp35-dependent mRNA isoforms, and conservation of significant mRNA isoforms in other filamentous fungi.

Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in *Cryptococcus neoformans*. Amanda L. Misener Bloom[1,2], Kurtis Downey[1], Nathan K. Wool[1], John C. Panepinto[1,2]. 1) Microbiology/Immunology, SUNY University at Buffalo, Buffalo, NY; 2) Witebsky Center for Microbial Pathogenesis and Immunology, SUNY University at Buffalo, Buffalo, NY.

In response to the hostile host environment, pathogens must undergo rapid reprogramming of gene expression to adapt to the stresses they encounter. Upon exposure to host temperature, Ribosomal protein (RP) transcripts are rapidly repressed in *C. neoformans*. We are interested in investigating specific mechanisms involved in this response, as this repression may be a critical process in host temperature adaptation. Using a mutant null of the major deadenylase, Ccr4, we have discovered that this repression is in part due to enhanced degradation of RP-transcripts. Ccr4 lacks a nucleic acid binding domain and therefore must be recruited to mRNA targets via RNA binding proteins. Using MEME analysis and chromatographic techniques, we have identified a shared cis element in the 3'UTR of RP transcripts that is recognized by the zinc knuckle protein, Gis2. We are currently investigating the importance of this protein-RNA interaction in the expression of RP genes.

Host temperature-induced enhanced degradation of RP transcripts is also dependent on the dissociable RNA polymerase II subunit, Rpb4. Specifically, we demonstrated that in an *rpb4*D mutant, RP-transcript deadenylation is impaired, suggesting that Rpb4 may be required for Ccr4-targeted degradation. In addition, we observed that upon a shift to 37°C, Rpb4 travels from the nucleus to the cytoplasm, supporting a role for Rpb4 in coupling transcription and degradation. Interestingly, this coupling is not restricted to the RP transcripts, as Rpb4 is also involved in enhanced decay of ER stress transcripts following their peak induction, one hour after a shift to host temperature. We have demonstrated that signaling through PKH enhances the degradation of the RP-transcripts in response to host temperature, but not the ER stress transcripts, highlighting the complexity of this system. We report that when transcription and degradation are uncoupled by the loss of Rpb4, growth at host temperature is impaired and virulence in a mouse model of disseminated cryptococcosis is attenuated. Our data suggests that coupling of transcription and degradation via Rpb4 allows the cell to control the intensity and duration of different responses at specific times following exposure to host temperature, contributing to the ability of *C. neoformans* to adapt to this stress.

Dual targeting of glycolytic enzymes by alternative splicing and translational read-through. Johannes Freitag, Julia Ast, Alina Stiebler, Michael Bölker. Department of Biology, Philipps-Universität Marburg, Marburg, Germany.

Processing of mRNA is a highly conserved process in eukaryotes involving three major steps. Nascent transcripts are capped at their 5'end, introns are removed by splicing and the 3'end is cleaved and polyadenylated. In the plant pathogenic fungus Ustilago maydis, several genes show hallmarks of differential splicing and alternative polyadenylation resulting in the production of C-terminally extended proteins. We detected that this process leads to generation of an extended glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoform harboring a C-terminal peroxisomal targeting sequence (PTS1). We could also detect peroxisomal isoforms of two further glycolytic enzymes, phosphoglycerate kinase (PGK) and triosephosphate isomerase (TPI). Remarkably, peroxisomal isoforms of PGK and TPI are generated by translational read-through in U. maydis. Further analysis revealed that dual targeting of glycolytic enzymes to peroxisomes and the cytoplasm is not restricted to U. maydis but occurs in a variety of fungal species. Interestingly, in different species variable mechanisms to generate extended peroxisomal isoforms of glycolytic enzymes are operating. In the ascomycete Aspergillus nidulans the PTS1-motif of PGK is derived from alternative splicing and polyadenylation, while translational read-through is used to generate a peroxisomal isoform of GAPDH. We could also show that some enzymes are partially targeted to peroxisomes by means of weak peroxisomal targeting signals. Dual localization of glycolytic enzymes to peroxisomes and the cytoplasm appears to be widespread in fungi. This indicates that fungal peroxisomes are endowed with a more complex metabolism than previously assumed. Thus, the consideration of alternative splicing and translational read-through will be of importance in future proteomic and metabolomic studies of organelles.

Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY. Mian Zhou[1], Jinhu Guo[5], Joonseok Cha[1], Michael Chae[1], She Chen[2], Jose Barral[3], Matthew Sachs[4], Yi Liu[1]. 1) Department of Physiology, UT Southwestern Medical Center, Dallas, TX; 2) National Institute of Biological Sciences, Beijing, China; 3) Departments of Neuroscience and Cell Biology and Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX; 4) Departments of Biology, Texas A&M University, College Station, TX; 5) School of Life Sciences, Sun Yat-sen University, Guangzhou, China.

Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation of highly expressed genes 1-3. In addition, codon usage is also implicated in the control of transcription, splicing and RNA structure 4-6. Many genes, however, exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. Alternatively, however, non-optimal codon usage may also have biological significance. The rhythmic expression and the proper function of the Neurospora FREQUENCY (FRQ) protein are essential for circadian clock function. Here, we show that, unlike most genes in Neurospora, frq exhibits non-optimal codon usage across its entire open reading frame. Optimization of frq codon usage results in the abolition of both overt and molecular circadian rhythms. Codon optimization not only increases FRQ expression level but surprisingly, also results in conformational changes in FRQ protein, impaired FRQ phosphorylation, and impaired functions in the circadian feedback loops. These results indicate that non-optimal codon usage of frq is essential for its circadian clock function. Our study provides an example of how non-optimal codon usage is used to regulate protein expression levels and to achieve optimal protein structure and function.

A transcriptome-wide view on microtubule-dependent mRNA transport. Carl Haag[1], Julian Konig[2], Kathi Zarnack[3], Michael Feldbrugge[1]. 1) Institut for Microbiology, Heinrich-Heine University, Düsseldorf, NRW, Germany; 2) MRC LMB Cambridge, UK; 3) EBI Hinxton, UK.

Long distance transport of mRNAs regulates spatio-temporal gene expression during polar growth. In filaments of *U. maydis*, for example, microtubule-dependent shuttling of mRNAs is crucial to determine the axis of polarity. The key component of this transport system is the RNA-binding protein Rrm4 that binds a distinct set of target mRNAs. Recently, we discovered a novel mechanism for mRNA transport, namely the co-transport of Rrm4 and associated mRNAs with endosomes. Here, new insights on mRNA transport will be presented using the improved in vivo UV-crosslinking technique: iCLIP. This technique allows identification of target mRNAs at the transcriptome-wide level with single nucleotide resolution.

Thursday, March 14 3:00 PM–6:00 PM
Kiln

Interactions between Fungi and Animals

Co-chairs: Neil Gow and Clarissa Nobile

Elicitation of host damage occurs in a temporally programmed manner during Aspergillus fumigatus infections. Elaine M. Bignell. Microbiology Section, Imperial College London, London, United Kingdom.

Background: In tissue-invasive lung infections caused by the mould Aspergillus fumigatus the molecular basis of host damage remains unclear. It has long been hypothesised that the secretion of proteolytic enzymes by invading A. fumigatus hyphae provides a mechanism by which epithelial damage is mediated. However, in whole animal studies of disease it has not been possible to substantiate an important role of fungal proteases since A. fumigatus mutants lacking individual or multiple enzyme functions retain the ability to cause fatal infections. One of the first cellular lines of defence against A. fumigatus infection is the monolayer of epithelial cells which line the mammalian airway. Epithelial cells provide a physical barrier against endothelial invasion and initiate an inflammatory immune response upon contact with A. fumigatus spores. Here we show that the A. fumigatus pH-responsive transcription factor, PacC, which governs expression of secreted proteases and secondary metabolism genes, is required for invasion of the murine pulmonary epithelium, and pathogenicity. Results: We determined, via murine and epithelial infection assays, that DpacC mutants are defective in elicitation of early-phase host damage which occurs, in wild type isolates, via a novel contact-dependent mechanism. Transcriptomic analyses of murine aspergillosis revealed aberrant cell wall biosynthesis in infecting DpacC isolates, suggesting a novel role for the A. fumigatus cell wall in pathogen-mediated host damage. Concordant with these findings PacC null mutants were shown to have signficiantly heightened chitin content in the fungal cell wall and were hypersensitive to cell wall perturbing agents, including caspofungin. The mechanistic relevance of cell wall-mediated host damage was verified by comparative analysis of damage elicited by cell wall extracts and heat-killed hyphae from wild type and DpacC isolates. Conclusion: A. fumigatus elicits host damage in a biphasic manner, initally via a novel contact-dependent mechanism involving cell wall components, and later via soluble mediators. A. fumigatus mutants deficient in the pH-responsive transcription factor PacC suffer deficits in both mechanisms. On the basis of this functional transcriptomic analysis we propose a new model of biphasic host damage during A. fumigatus infections.

Exploiting innate recognition of fungi for vaccine development. Stuart Levitz. Medicine, University of Massachusetts, Worcester, MA.

Most licensed vaccines work by promoting protective antibody responses. However, some populations, such as the elderly and the immunocompromised, generally have poor antibody responses to conventional vaccines. Moreover, for many infectious and neoplastic diseases, vaccines that arm adaptive T cell responses appear necessary. Thus, a major challenge in vaccinology is the development of platforms and adjuvants that effectively promote protective T cell and antibody responses. The immune system has evolved to innately recognize components of the fungal cell wall, particularly b-glucans. Research in my laboratory, in collaboration with Gary Ostroff, has focused on how this innate recognition of the fungal cell wall can be exploited for vaccine development. To achieve this aim, we have used glucan particles (GPs) as a novel vaccine platform. GPs are hollow, highly purified microcapsules prepared from *Saccharomyces cerevisiae* cell walls. GPs are composed predominantly of b-1,3-glucan and are recognized by b-glucan receptors (particularly Dectin-1) on dendritic cells and other phagocytes. GPs also potently activate complement, resulting in opsonization and recognition by complement receptors. GPs can be loaded with antigens and immunomodulators such that the “payload” is released following phagocytosis. We have demonstrated robust and long-lasting antigen-specific T cell (Th1- and Th17-biased) and antibody responses following immunization of mice with GPs “encapsulated” with antibody. Moreover, vaccination of mice with GPs loaded with fungal antigens can protect mice against lethal challenges with the pathogenic fungi *Cryptococcus neoformans* and *Histoplasma capsulatum*.

Regulatory circuits governing Candida albicans proliferation in a mammalian host. Jose C. Perez[1], Carol A. Kumamoto[2], Alexander D. Johnson[1]. 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) Molecular Biology and Microbiology, Tufts University, Boston, MA.

The fungus Candida albicans resides in the gastrointestinal tract of most, if not all, human adults and is also a leading cause of life-threatening fungal infections in immunocompromised individuals. C. albicans has no known environmental reservoir suggesting that it has extensively co-evolved to thrive in its host. To uncover the C. albicans gene circuits governing its proliferation in a host, we used mouse models of intestinal colonization and systemic infection to screen a set of ~75 transcription regulator deletion strains. These mutant strains were chosen because they showed no gross phenotypes when cultured under a variety of laboratory growth conditions. We identified eight transcription regulators that play roles in intestinal colonization, systemic infection or both. Through genome-wide chromatin immunoprecipitation and transcriptional profiling experiments, we determined the target genes and the general circuitry controlled by these regulators. Our results reveal multiple biological functions necessary for C. albicans to inhabit a mammalian host, the acquisition of carbon and nitrogen sources being prominent among them. These findings highlight common challenges faced by bacterial and eukaryotic (fungal) species when colonizing the mammalian intestine and illustrate how evolution has tinkered with the C. albicans regulatory circuitry to meet these demands.

Dramatic ploidy change as an adaptive strategy in Candida albicans... Meleah A. Hickman, Ben Harrison, Darren Abbey, Anja Forche, Carsten Paulson, Kathleen Matter, Judith Berman. Dept Gen, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN.

For over 100 years, Candida albicans has been considered an obligate diploid, although it clearly tolerates single chromosome aneuploidy as well as long tracts of homozygosity. We recently identified tetraploid, triploid as well as intriguing reductions to below diploid C. albicans cells, some from the clinic, others from a mouse host and others following stress exposure in vitro. Tetraploidy arises either through parasex (mating between diploid cells) or defects in mitosis. Stress conditions, including exposure to the antifungal drug flucanozole, increase the frequency of tetraploid formation. The polyploid state is relatively unstable even under standard laboratory conditions and loss of a heterozygous marker increases by an order of magnitude as compared to diploid populations. A small subset of tetraploid cells return to a near diploid state very rapidly even without exposure to the stresses usually used to induce concerted chromosome loss. The diploid derivatives of polyploid cells exhibit a wide range of chromosome aneuploidies and homozygosities, thus generating a wide range of genetic diversity within a single population. Evolution experiments with fluconazole suggest that diploid cells undergo transient polyploidization in response to fluconazole and that polyploid cells adapt to stress conditions more rapidly.

Nematode-trapping fungi eavesdrop on nematode pheromones. Yen-Ping Hsueh[1], Parag Mahanti[2], Frank Schroeder[2], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Inst of Technology, Pasadena, CA; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

The recognition of molecular patterns associated with specific pathogens or food sources is fundamental to ecology and plays a major role in the evolution of predator-prey relationships. Recent studies showed that nematodes produce an evolutionarily highly conserved family of small molecules, the ascarosides, which serve essential functions in regulating nematode development and behavior. Here we show that nematophagous fungi, natural predators of soil-dwelling nematodes, can detect and respond to ascarosides. Nematophagous fungi use specialized trapping devices to catch and consume nematodes, and previous studies demonstrated that most fungal species do not produce traps constitutively but rather initiate trap-formation in response to their prey. We found that ascarosides, which are constitutively secreted by many species of soil-dwelling nematodes, represent a conserved molecular pattern used by nematophagous fungi to detect prey and trigger trap formation. Ascaroside-induced morphogenesis is conserved in several closely related species of nematophagous fungi and occurs only under nutrient-deprived condition. Our results demonstrate that microbial predators eavesdrop on chemical communication among their metazoan prey to regulate morphogenesis, providing a striking example of predator-prey co-evolution. We anticipate that these findings will have broader implications for understanding other inter-kingdom interactions involving nematodes, which are found in almost any ecological niche on Earth.

A morphogenesis regulator controls cryptococcal neurotropism. Xiaorong Lin[1], Bing Zhai[1], Karen Wozniak[2], Srijana Upadhyay[1], Linqi Wang[1], Shuping Zhang[3], Floyd Wormley[2]. 1) Biology, Texas A&M University, TAMU-3258, TX; 2) Biology, the University of Texas at San Antonio, San Antonio, Texas, USA; 3) Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA.

Cryptococcus neoformans is the major causative agent of cryptococcal meningitis, a disease that is responsible for more than 600,000 deaths each year. This ubiquitous environmental pathogen enters host lungs through inhalation and typically establishes asymptomatic latent infections. However, extrapulmonary dissemination often occurs in individuals with weakened immunity and Cryptococcus has a predilection to infect the brain. Brain infections are fatal and formidable to treat due to the poor penetration of most antifungals to the brain. Unfortunately, little is known about cryptococcal factors that control its neurotropism. Here we report that a morphogenesis regulator Znf2 controls the tissue tropism of cryptococcal infection. In particular, activation of Znf2 abolishes Cryptococcus extrapulmonary dissemination and consequently leads to the absence of fatal brain infections in the inhalation infection model. Although Znf2 overexpression strains are avirulent in this animal model, these strains are capable of proliferating in the animal lungs during the early stages of infections. Histological examinations and cytokine profiling revealed that the Znf2 overexpression strain causes enhanced monocyte infiltration in the animal lungs. Consistently, the Znf2 overexpression strain stimulates pro-inflammatory host responses while suppresses deleterious Th2 host responses during early stage of infection in the pulmonary infection model. Such protective host defense responses might have prevented the extrapulmonary dissemination of Cryptococcus. In the intravenous infection model where the lung infection was bypassed and there was uniform hematogenous dissemination, the Znf2 overexpression strain showed a specific defect in the brain infection. Taken together, our data indicate that Znf2 helps polarize the host immunity towards protection and that it mediates cryptococcal tissue tropism during infection.

Sit and wait: Special features of *Aspergillus terreus* in macrophage interactions and virulence. M. Brock[1], I.D. Jacobsen[2]. 1) Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knoell Institute, Jena, Germany; 2) Molecular Pathogenicity Mechanisms, Hans Knoell Institute Jena, Germany.

While *Aspergillus fumigatus* is known as the main cause of invasive pulmonary aspergillosis in immunocompromised patients, *Aspergillus terreus* is an emerging pathogen prevalent in some local hot spots. When tested in embryonated egg or murine infection models *A. terreus* required substantially higher infectious doses compared to *A. fumigatus* to cause high mortality rates. Furthermore, when *A. fumigatus* and *A. terreus* infections were followed by *in vivo* imaging using bioluminescent reporter strains, germination and tissue invasion of *A. terreus* was significantly delayed. To elucidate differences in more detail, the interaction of *A. terreus* and *A. fumigatus* with macrophages was compared. *A. terreus* was phagocytosed significantly faster, which appears mainly due to higher exposure of galactomannan and glucans on the surface of conidia. Additionally, although phagocytosis of both species resulted in phagolysosome maturation, *A. fumigatus* efficiently inhibited acidification, which was not the case for *A. terreus*. However, within this acidic environment of phagolysosomes *A. terreus* showed long-term persistence without significant inactivation of conidia. Further analyses revealed that inefficient blocking of acidification by *A. terreus* was due to differences in the spore colour pigment of both species. Recombinant production of a naphthopyrone synthase from *Aspergillus nidulans* enabled *A. terreus* to inhibit the acidification to a similar extent as observed for *A. fumigatus*. This alteration of the phagolysosomal environment resulted in an increased escape from macrophages and was accompanied by increased virulence in a murine infection model. We speculate that the long-term persistence of *A. terreus* wild-type strains in acidified phagolysosomes might be responsible for high dissemination rates observed in infected human patients, because *A. terreus* might hitchhike inside immune effector cells to reach secondary sites of infection.

The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans. Jason Funt[1], Darren Abbey[7], Luca Issi[5], Brian Oliver[3], Theodore White[4], Reeta Rao[5], Judith Berman[6], Dawn Thompson[1], Aviv Regev[1,2]. 1) Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142; 2) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Masscahusetts Ave, Camridge, MA 02140; 3) Seattle Biomedical Research Institute, Seattle, WA; 4) School of Biological Sciences, University of Missouri at Kansas City, MS; 5) Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester MA 01609; 6) Tel Aviv University, Ramat Aviv, 69978 Israel; 7) University of Minnesota, Minneapolis MN 55455 USA.

Candida albicans is both a member of the healthy human microbiome and a major pathogen in immunocompromised individuals1. Infections are most commonly treated with azole inhibitors of ergosterol biosynthesis. Prophylactic treatment in immuncompromised patients2,3 often leads to the development of drug resistance. Since C. albicans is diploid and lacks a complete sexual cycle, conventional genetic analysis is challenging. An alternative approach is to study the mutations that arise naturally during the evolution of drug resistance in vivo, using isolates sampled consecutively from the same patient. Studies in evolved isolates have implicated multiple mechanisms in drug resistance, but have focused on large-scale aberrations or candidate genes, and do not comprehensively chart the genetic basis of adaptation5. Here, we leveraged next-generation sequencing to systematically analyze 43 isolates from 11 oral candidiasis patients, collected sequentially at two to 16 time points per patient. Because most isolates from an individual patient were clonal, we could detect newly acquired mutations, including single-nucleotide polymorphisms (SNPs), copy-number variations and loss of heterozygosity (LOH) events. Focusing on new mutations that were both persistent within a patient and recurrent across patients, we found that LOH events were commonly associated with acquired resistance, and that persistent and recurrent point mutations in over 150 genes may be related to the complex process of adaptation to the host. Conversely, most aneuploidies were transient and did not directly correlate with changes in drug resistance. Our work sheds new light on the molecular mechanisms underlying the evolution of drug resistance and host adaptation.

Thursday, March 14 3:00 PM–6:00 PM
Nautilus

Fungal Volatiles and Organic Compounds as Signaling Agents

Co-chairs: Joan Bennett and Richard Splivallo

Fungi reacting to rhizobacterial volatiles. Birgit Piechulla, Piyali Das, Uta Effmert. University of Rostock, Rostock, Germany.

Microorganisms, similar as other organisms are able to synthesize and release volatile organic compounds (VOCs), which are responsible for characteristic blends or aromas of for example foodstuff such as wine and cheese as well as spoiled meat. The capability of microorganisms to emit complex volatile mixtures is tremendous. More than 800 volatiles are presently known that are emitted by microorganisms (database of volatiles of microorganisms DOVE-MO). Beside the wealth of volatile emissions, to date not much is known about the biological functions of these compounds. To study volatile-mediated interactions of plant associated bacteria and fungi, various rhizobacteria and phytopathogenic fungi were co-cultivated in bipartite Petri dishes, which allow only volatiles to traverse from one to the other compartment. The volatiles of Serratia, Stenotrophomonas, Pseudomonas, Burkholderia and Staphylococcus inhibited the growth of Aspergillus, Fusarium, Microdochium, Neurospora, Rhizoctonia, Phaecilomyces, Penicillium, Phoma, Sclerotinia, Trichoderma and Verticillium in species specific manner. The reactions of Sclerotinia scleotiorum to Serratia sp. 4Rx13 volatiles were studied in more detail, e.g. radial growth, biomass formation, catalase activity and lipid peroxidation. Furthermore, the volatile mixture of Serratia sp. 4Rx13 was studied using headspace collection systems and GCMS analysis. Ca. 100 volatiles were separated, some of them were identified, most of them remain unknowns or structures have to be elucidated. References: Kai et al. (2007) Arch. Microbiol. 187:351-360 Vespermann et al. (2007) Appl. Environ. Microbiol. 73:5639-5641 Kai et al. (2010) Appl. Microbiol. Biotechnol. 88:965-976 Effmert et al. (2012) Chem. Ecol. 38:665-703.

Enhancement of plant growth and stress resistance by Fusarium volatile organic compounds: A novel mechanism mediating plant-fungal interactions. Seogchan Kang[1,3], Vasileios Bitas[1,3], Nate McCartney[2,3], Jim Tumlinson[2,3]. 1) Plant Pathology & Environmental Microbiology, Pennsylvania State Univ, University Park, PA; 2) Entomology, Pennsylvania State Univ, University Park, PA; 3) Center for Chemical Ecology, Pennsylvania State Univ, University Park, PA.

Every organism employs an elaborate network of signaling pathways for sensing stimuli from surrounding environments and neighboring organisms and translating them into specific molecular and cellular responses. Production and perception of a vast array of secreted proteins and metabolites plays key roles in this mechanism. A group of secreted molecules that are ubiquitous but often overlooked is volatile organic compounds (VOCs). VOCs can travel far from their point of production through the atmosphere as well as porous soils, making them ideal signaling molecules for mediating organismal interactions without physical contact. Roles of animal- and plant-derived VOCs in directing animal behaviors and roles of plant VOCs in chatters of “talking trees” are well known and serve critical roles in diverse ecological processes. In contrast, the available knowledge of microbial VOCs as semiochemicals is limited and mostly circumstantial. Multiple isolates of *Fusarium oxysporum*, a soil-borne, cosmopolitan fungus that often resides in the rhizosphere of many plants, produce unknown VOCs that drastically enhance the growth and stress resistance of *Arabidopsis thaliana*. Other *Fusarium* species also promoted *Arabidopsis* growth. Molecular and cellular changes underpinning the *Fusarium* VOC-mediated signaling will be discussed. Given the vast diversity of fungi in nature and the critical importance of fungal communities for the ecology and fitness of plants, VOC-mediated signaling is a mostly uncharted frontier, waiting for systematic exploration.

The Role of Quorum-sensing Molecules in Interactions between *Candida albicans* and its Host. Jessica C. Hargarten[1], Thomas M. Petro[2], Kenneth W. Nickerson[1], Audrey L. Atkin[1]. 1) School of Biological Sciences, University of Nebraska, Lincoln, Lincoln, NE; 2) Department of Oral Biology, University of Nebraska Medical Center, Lincoln, NE.

Candida albicans is a polymorphic fungus that is capable of causing the life threatening disease Candidiasis once it reaches the bloodstream of a susceptible host. The capability to switch between morphologies, and its ability to synthesize and secrete the quorum sensing molecule (QSM) farnesol are known virulence factor. Previously, we showed that *C. albicans* mutants that produced less farnesol are less pathogenic to mice than their parental strain in a tail vein assay. Also, oral administration of farnesol to the mice prior to infection increased mortality. In contrast, farnesol blocks the yeast to mycelia transition in vitro, which should have a protective effect. These observations pose the dilemma of finding a mechanism whereby a molecule which blocks the yeast to mycelia transition can also act as a virulence factor. We hypothesize that farnesol functions as a virulence factor by modulating the host innate immune response. Distinct *Candida* morphologies elicit different host immune responses. Both white and opaque cells stimulate leukocyte movement, but only white cells secrete a small molecular weight chemoattractant that draws the leukocyte directly towards the white cell and stimulates engulfment by mouse macrophages. The white cells are also less susceptible to killing by human macrophages and neutrophils than opaque cells, possibly due to their increased capabilities of escape once phagocytosed. The chemical identity of this chemoattractant is currently unknown, but the reason behind its continued secretion by white cells is intriguing. One likely candidate is farnesol because opaque cells, unlike white cells, do not accumulate detectable levels of farnesol. Macrophages are capable of detecting and responding to exogenous farnesol. Earlier our group reported that farnesol stimulates the expression of both pro-inflammatory and regulatory cytokines by mouse macrophage. The production of these warning signals is an important indicator of how the body ultimately hopes to clear the infection. Others have shown that farnesol suppresses the anti-*Candida* activity of macrophages through its cytotoxic effects, thus making it all the more difficult to eliminate the fungus early in infection. Here we report the in vitro role of farnesol and other known QSM in macrophage chemotaxis and relative phagocytosis of *C. albicans*.

Innate Immunity in *Fusarium graminearum*. Vong shian Simon Ip Cho[1,2], Gitte Erbs[3], Thomas Sundelin[3], Peter Busk[4], Mari-Anne Newman[3], Stefan Olsson[1]. 1) Genetics and Microbiology, University of Copenhagen, Copenhagen, Denmark; 2) USDA-ARS Cereal Disease Laboratory, University of Minnesota, Saint Paul, MN, USA; 3) Transport Biology, University of Copenhagen, Copenhagen, Denmark; 4) Dept. Biotechnology, Aalborg University, Copenhagen, Denmark.

Fungi are often mostly recognized as plant pathogens that cause harm to important economical plants. In nature however, fungi are frequently victims of bacterial parasitism but little is known about fungal defense mechanisms. The potential existence of fungal innate immunity was studied using *Fusarium graminearum* as model organism and bacterial flagellin to mimic the presence of bacteria in an *in vitro* environment. The presence of flagellin triggered an initial mitochondrial and cell membrane hyperpolarization which was detected using the florescent dye $DiOC_7(3)$. This was followed by the production of the secondary signalling molecule Nitric Oxide (NO), common to innate immunity signalling in other eukaryotes. NO was monitored using the fluorescent dye DAF-FM. NO appears to be produced by an inducible enzyme that is regulated by complex mechanisms but centrally modulated by Calcium/Calmodulin. Inhibition studies suggest the presence of a Nitric Oxide Synthase (NOS), but no typical arginine utilizing NOS was identified within the *F. graminearum*'s genome by homology search. Various genes bearing resemblance to the archetypal NOS, as well as argininosuccinate lyase were deleted. However, the mutants still produced NO. The presence of alternative pathways contributing towards the production of NO was investigated by adding a variety of potential substrates to challenged cultures. Various reactions were observed suggesting that several pathways are present. In conclusion, *F. graminearum* reacts strongly to the presence of the bacterial Microbial Associated Molecular Pattern (MAMP) flagellin with an up-regulation of NO production showing the presence of innate immunity-like responses also in fungi.

The *Trichoderma reesei* polyketide synthase gene *pks1* is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness. Lea Atanasova[1], Benjamin P. Knox[2], Christian P. Kubicek[1], Scott E. Baker[2], Irina S. Druzhinina[1]. 1) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; 2) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, Richland, WA, USA.

The economically important genus *Trichoderma* (Hypocreales, Ascomycota, Dikarya) is well known for its mycotrophic lifestyle and for the broad range of biotrophic interactions with plants and animals. Moreover it contains several cosmopolitan species characterized by their outstanding environmental opportunism. These properties have given rise to the use of several species in agriculture as biopesticides and biofertilizers while *T. reesei* is applied for production of bioenergy-related enzymes. The molecular basis of the opportunistic success of *Trichoderma* is not yet well understood. While there is some evidence for a role of secreted enzymes and proteins, less is known about a possible role of secondary metabolites. Recently it was predicted that the PKS encoding gene *pks1* from *T. reesei* and its orthologues are most likely responsible for the characteristic yellow-green pigmentation of conidia. To reveal the full function of the gene we deleted it from the wild-type strain QM 6a what resulted in complete loss of the green coloration of conidia. The ecophysiological profiling of *Dpks1* showed that the gene is also involved in multiple functions at different stages of the *T. reesei* life cycle. Testing the antagonistic antifungal potential of the *T. reesei Dpks1* mutant against several host/prey fungi suggested that the loss of *pks1* reduced the ability to combat them by means of both mechanisms: the pre-contact inhibition and direct overgrowth. However the overall analysis of mycoparasitic interactions suggests that the gene is most likely involved in protection against other fungi rather than in attacking them. Interestingly, we noticed the increased production of volatile compounds by the *Dpks1* strains. The phenotype microarrays showed that PKS1 encoding gene restricts *T. reesei* from conidiation on a number of the best utilized carbon sources but does not influence the sexual development except the alteration of stromata pigmentation. The data for transcriptional response of genes putatively involved in above mentioned processes will be presented.

Semiochemicals and signaling: plant responses to *Trichoderma* volatile organic compounds. Richard Hung. Plant Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Volatile organic compounds (VOCs) produced by *Trichoderma viride* have recently been shown to have plant growth promoting effects on *Arabidopsis thaliana*. This finding adds a new facet to the multiple methods which fungi in the genus *Trichoderma* promote plant health and are beneficial to humans. Both above and below ground growth was greater in *A. thaliana* exposed to naturally produced *T. viride* VOCs as compared to controls. The average root mass of control plants was 0.36g and the average mass of VOC exposed plants was 0.77g showing a 113% increase in plant mass. In addition there was a 60% increase in chlorophyll concentration (5.5mg/g control, 8.8mg/g test). GCMS analysis of the VOCs produced by *T. viride* has resulted in 51 identified compounds. Several compounds from the GCMS data were chosen to determine the effects of individual compounds on the health of *A. thaliana*. The compound trans-2-octenol at concentrations of 1ppm caused decreased dry weight (14% less than control) and extended root length (16% longer than control), indicative of stress. At 1 and 10ppm, the compound 2,5-dimethylfuran, which has been reported to be produced by *Trichoderma* but was not found in the aforementioned GCMS analysis, caused only visual differences. The exposed *A. thaliana* had extended stems as compared to controls but no other differences. In summary, the individual compounds of the *T. viride* volatile profile that were tested, did not promote plant growth.

Identification of chemoattractant compounds from tomato root exudate that trigger chemotropism in *Fusarium oxysporum*. El Ghalid Mennat, David Turra, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain.

Fusarium oxysporum is a soilborne pathogen that causes vascular wilt disease on a wide range of plant species, including tomato (*Solanum lycopersicum*). The host signals that trigger fungal infection are currently unknown. A chemotropic response of *F. oxysporum* towards tomato root exudate was observed using a plate assay that measures directed growth of fungal germ tubes towards chemoattractants. To purifiy the chemoattractant coumpound(s) from tomato root exudate, we applied a series of purification methods including extraction with organic and inorganic solvents, fractionation by size exclusion and ion exchange chromatography. The compound(s) showing chemoattractant activity were found in the hydophilic fraction, had a molecular weight between 30 and 50 kDa and were sensitive to boiling and treatment with proteinase K, suggesting that they correspond to one or several secreted tomato proteins. Polyacrylamide gel electrophoresis of the active fraction revealed multiple protein bands of the expected size, two of which displayed chemoattractant activity when eluted from the gel. Identification of the active protein(s) by LC-ESI-MS is currently ongoing. Identification of the secreted chemoattractant(s) from tomato roots will advance our understanding of the molecular events that trigger fungus-root interactions.

The mixed fungal and bacterial origin of truffle aroma. Richard Splivallo[1], Aurélie Deveau[2], Nayuf Valdez[1], Nina Kirchhoff[1], Pascale Frey-Klett[2], Petr Karlovsky[1]. 1) Molecular Phytopathology and Mycotoxin Research, Georg-August University of Goettingen, Grisebachstrasse 6, Germany; 2) UMR1136 INRA Université de Lorraine "Interactions Arbres/Micro-organismes", Labex ARBRE, IFR110 EFABA, Centre INRA of Nancy, 54280 Champenoux, France.

Truffles are symbiotic ectomycorrhizal fungi which develop on plant roots. Their fruiting bodies are highly appreciated by humans for their aroma, which typically comprises twenty to fifty volatiles per truffle species. The biosynthetic routes leading to characteristic truffle volatiles have not yet been fully characterized. By similarity to yeasts, volatile synthesis in truffles most likely involves amino acid and fatty acid catabolism. Truffle fruiting bodies further contain a diverse microbial community which might be able to generate volatiles or biotransform volatile-precursors on its own. Our aim was to investigate the formation of sulphur containing volatiles in truffles, because sulphur volatiles play a major role in the truffle ecology and are determinant of their quality (for humans). We demonstrate that sulphur volatiles characteristic of the white truffle *T. borchii* are actually produced by bacteria colonizing truffle fruiting bodies. Under laboratory bioassays, sulphur containing compounds (thiophenes volatiles) resulted from the transformation by bacteria of non-volatile precursor(s) into volatiles. Interestingly in our assays thiophene volatiles were detectable only from bacteria and not from truffle mycelium, while other compounds such as dimethyl disulfide were detectable from both organisms. This indicates that some volatiles might be produced by both truffle mycelium and bacteria, but thiophene volatiles most likely originate from bacteria only. Characterization of the bacterial population by Fluorescence In Situ Hybridization highlighted that the concentration of thiophene volatiles correlated with the bacterial density inside fruiting bodies. This gives further ground to the bacterial origin of thiophene volatiles. Additionally the production of thiophene volatiles was suppressed upon treating truffle fruiting bodies with antibacterial or antifungal agents, suggesting that the precursors of thiophene volatiles might be synthesized by both fungi and bacteria. These unexpected results disprove the earlier belief that truffles were able to synthesize their aroma on their own. They add a new dimension to plant-fungal interactions by highlighting the importance of the bacterial community associated to truffle fruiting bodies.

Thursday, March 14 3:00 PM–6:00 PM
Scripps

Genomics and Biochemistry of Degradation of Complex Molecules in the Environment

Co-chairs: Jonathan Walton and Dan Cullen

Fungal transcriptome as database for proteome and refinement tool of gene annotation. K. Igarashi[1], C. Hori[1], M. Ishiguro[1], Y. Uemura[2], A. K. Takeda[2], S. Kaneko[3], M. Samejima[1]. 1) University of Tokyo, Tokyo, Japan; 2) Genaris, Inc., Kanagawa, Japan; 3) National Food Research Institute, Ibaraki, Japan.

So far, wood-rotting basidiomycetes, such as white-rot and brown-rot fungi, are the organisms known to grown on wood. They produce various enzymes to outside of their cell, extracellular part of the mycelia, in order to degrade major components of plant cell wall such as cellulose, hemicellulose and lignin. There are many enzymes, which can be utilized for the biomass conversion, in those fungi, as well as the proteins helping and/or accelerating the degradation of the plant cell wall. Therefore, combination of correct annotation of these genes and the proteome analysis of the extracellular enzymes are quite important for biomass utilization. In the present study, we have cultivated the white-rot basidiomycetes *Flammulina velutipes* (Enoki-take, winter mushroom) and *Phanerochaete chrysosporium* in various biomass-degrading culture, and the transcriptome databases were constructed by sequencing of the cDNA library using 454 sequencer. In *F. velutipes*, we identified 19 novel biomass-degrading enzymes including 12 carbohydrate-active enzymes (CAZymes) by 2-dimentional gel electrophoresis of extracellular proteins from cellulose-grown culture, using the transcriptome data as a reference sequence. In the case of *P. chrysosporium*, the transcriptome sequence data was also used to improve the gene annotation, and more than 1,000 genes are newly annotated by the algorithms refined by cDNA sequences. The improvement of gene annotation caused accurate prediction of introns and showed unique monodispersed distribution of intron length in this fungus.

Developmental regulation and cellulase gene expression in *Trichoderma reesei*. Irina S. Druzhinina[1,2], Razieh Karimi-Aghcheh[1], Lea Atanasova[1], Christian P. Kubicek[1,2]. 1) Microbiology Group, Institute of Chemical Engineering, Vienna, Austria; 2) Austrian Center of Industrial Biotechnology, c/o Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria.

We have recently shown that expression of cellulase and hemicellulase encoding genes in *Trichoderma reesei* (teleomorph *Hypocrea jecorina*) is obligatorily dependent on the function of the protein methyltransferase LAE1. Its orthologue in *Aspergillus nidulans*, LaeA, is a part of the VELVET protein complex consisting of LaeA, VeA and VelB that regulates secondary metabolism and sexual reproduction. Here we have investigated a possible role of VEL1, the *T. reesei* orthologue of *A. nidulans* VeA, in expression cellulase genes and the development of the fungus. The *T. reesei vel1* gene is not expressed in the darkness and is expressed at a relatively low level under illumination. Deletion of the *vel1* locus causes a complete loss of conidiation and essential alteration in sexual development such as loss of formation of perithecia. Overexpression of *vel1* under the constitutive expression signals of *tef1* did not enhance conidiation in light or darkness. However it led to irregular formation of infertile perithecia in the darkness. Deletion of *vel1* did not affect cellulase gene expression, but *vel1* overexpression strongly enhanced it. Consistent findings were also obtained for expression of xylanase and b-xylosidase genes. The stimulation of cellulase gene expression by overexpressing *vel1* was dependent on a functional *lae1* gene. Our data show that VEL1 controls photoinduced sexual development and light-independent conidiation. In addition, while *vel1* overexpression stimulates cellulase gene expression, is dispensable for this process and its action is therefore different from that of LAE1.

Parallel losses of genes associated with saprotrophy in ectomycorrhizal Agaricomycotina lineages. D. Floudas[1], L. Nagy[1], A. Kohler[2], A. Kuo[3], I. Grigoriev[3], F. Martin[2], D. Hibbett[1]. 1) Biology, Clark Univ, Worcester, MA; 2) Lab of Excellence ARBRE, Tree-Microbes Department, INRA-Nancy, 54280 Champenoux, France; 3) DOE Joint Genome Institute, Walnut Creek, CA.

Mushroom forming fungi (Agaricomycotina) play pivotal roles in the cycling of nutrients in terrestrial ecosystems. Agaricomycotina exhibit diverse lifestyles including saprotrophs and symbionts, such as mutualistic ectomycorrhizas. Previously, as part of the Saprotrophic Agaricomycotina Project (SAP), we performed analyses of fungal genomes focusing on wood decayers, which suggested that white rot is the plesiomorphic nutritional strategy of Agaricomycetes and emerged 300 million years ago at the end of Carboniferous era. Our analyses also suggested that the brown rot mechanism and the mycorrhizal lifestyle of *Laccaria bicolor* have emerged from white rot ancestors. The transitions from white rot to brown rot have taken place several times in Agaricomycotina and were accompanied by losses of genes encoding enzymes involved in lignin and crystalline cellulose degradation. A similar pattern was reconstructed for the transition from a saprotrophic towards a mycorrhizal lifestyle in *L. bicolor*, which was the first mycorrhizal species in the Agaricomycotina to have its genome sequenced. However, *L. bicolor* represents only one of many ectomycorrhizal lineages recognized across Agaricomycotina. Here, we present data from eleven newly sequenced mycorrhizal genomes of Agaricomycotina, generated under the auspices of the Mycorrhizal Genomes Initiative (MGI), in addition to 8 new genomes of decayers from the SAP. With these new genomes in hand, we are able to explore how the emergence of mycorrhizal lifestyles is associated with changes in numbers of genes encoding enzymes involved in degradation of plant biopolymers. The results suggest that ectomycorrhizal lifestyles have emerged multiple times from both white rot and brown rot ancestors in Agaricomycotina. The transitions to the ectomycorrhizal lifestyle show parallelism in gene losses between *L. bicolor* and other mycorrhizal Agaricomycotina lineages. However, patterns of retention of genes encoding lignocellulolytic enzymes vary across ectomycorrhizal lineages. For example, cellobiohydrolases, which are involved in the degradation of crystalline cellulose, have been retained in several mycorrhizal lineages. The results suggest that the emergence of ectomycorrhizal lineages in Agaricomycotina has been associated with different degrees of reduction of their saprotrophic ability.

Co-expression analysis of *Phanerochaete carnosa* during growth on hardwood and softwood species to predict proteins with unknown function relevant to biomass conversion. Hitoshi Suzuki[1], Chi Yip Ho[2], Kin Chan[2], Philip Wong[1], Yunchen Gong[1], Elisabeth Tillier[1], Emma Master[1]. 1) University of Toronto, Toronto, Ontario, Canada; 2) Mount Sinai Hospital, Toronto, Ontario, Canada.

Softwood is the predominant form of land plant biomass in the Northern hemisphere and is among the most recalcitrant biomass resource to bioprocess technologies. The white rot fungus *Phanerochaete carnosa* has been isolated almost exclusively from softwoods, while most known white-rot species, including the model fungus *Phanerochaete chrysosporium*, were mainly isolated from hardwoods. Growth studies of *P. carnosa* and *P. chrysosporium* on sapwood and heartwood from deciduous and coniferous species revealed comparable growth of *P.carnosa* on all wood samples, while *P. chrysosporium* grew poorly on heartwood from conifers. A contributing factor to growth on extractive-rich heartwood samples could be the comparatively high number of P450 monooxygenases encoded by *P. carnosa*. Notably, genome sequencing revealed that *P. carnosa* possesses one of the largest P450 contingents (239 P450s) among the sequenced and annotated wood-rotting basidiomycetes. However, like most sequencing efforts, a significant fraction of the *P. carnosa* genome comprises genes that encode proteins with unknown function. Moreover, transcripts from several of these genes were identified in mycelia collected at a single time point from *P. carnosa* cultivations growing on woody biomass. Accordingly, the aim of the current study was to analyze co-expression patterns of known and unknown genes to identify those with unknown function that might be most relevant to biomass conversion. Our approach was to separately cultivate *P. carnosa* on ball-milled trembling aspen (*Populus tremuloides*) and ball-milled white spruce (*Picea glauca*) and to collect mycelia at five time points over a one-month cultivation period. RNA collected from all cultures at each time point was sequenced separately using the Illumina HiSeq platform. Co-expression patterns will be described and used to predict new gene products that are particularly interesting to target for detailed biochemical characterization.

Functional Analysis of the *Pleurotus ostreatus* Manganese-Peroxidase Gene Family. Tomer Salame, Doriv Knop, Dana Levinson, Oded Yarden, Yitzhak Hadar. Microbiology and Plat Pathology, Hebrew Unversity, Rehovot, Israel.

Mn amendment to *P. ostreatus* cultures enhances degradation of recalcitrant aromatic compounds. Manganese peroxidase (MnP) isoenzymes are key players in these processes. The MnP gene family is comprised of five Mn -dependent peroxidases (mnp3, 6, 7, 8 and 9) and four versatile-peroxidases (mnp1, 2, 4 and 5; VPs). In liquid medium, Mn amendment resulted in a drastic up-regulation of the predominantly expressed mnp3 and mnp9, and down-regulation of mnp4. To obtain direct evidence for the role of these enzymes, we produced genetically-modified (knockout, knockdown and/or over-expression) strains in *mnps* and studied their degradation capacity. The compounds studied were: azo-dyes such as orange II and reactive black, recalcitrant pharmaceutical compounds found in treated waste water such as Carbamazepine and lignocellulosic agricultural waste. We engineered a transformant, constitutively expressing mnp4 a VP naturally repressed by Mn (designated OEmnp4) under the control of the b-tubulin promoter. Now, despite the presence of Mn in the medium, OEmnp4 produced mnp4 transcript as well as VP activity as soon as four days after inoculation. OEmnp4 decolorized the azo-dyes two days earlier relative to the wild type in Mn amended medium. RNAi silencing targeting mnp3 resulted in a delay in the decolorization capacity which occurred concomitantly along with a marked reduction of the expression level of all mnps, particularly mnp3 and mnp9. This observation supported the conclusion that MnPs are involved in the process but could not determine the specific contribution of the different genes to the outcome. Therefore we produced a Dku80 strain, exhibiting a 100% homologous DNA recombination rate, to enable specific gene replacement. Subsequently, homokaryon mnp2, 3, 4 and 9 knockout strains were produced. In Mn amended GP, orange II decolorization was not significantly inhibited by any of these strains, indicating on functional redundancy. In Mn deficient GP, inactivation of mnp4 proved that it encodes the key VP responsible for Mn dependent and Mn independent peroxidase activity, as well as resulted in reduction of the azo dye reactive black 5 decolorization capacity. The tools and protocols developed increase the amenability of *P. ostreatus* to genetic manipulations and expand options for gene function analyses.

Carbon source and light dependent regulation of gene clusters in *Trichoderma reesei* (*Hypocrea jecorina*). Doris Tisch[2], Monika Schmoll[1]. 1) Health and Environment, Bioresources, Austrian Institute of Technology AIT, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria.

Trichoderma reesei (anamorph of Hypocrea jecorina) is one of the most prolific producers of plant cell wall degrading enzymes. Regulation of the genes encoding these enzymes occurs in response to the nutrient sources available in the environment and many of them are responsive to light as well. Cellulose as the natural substrate induces the most complete enzyme set, while induction of cellulases also occurs on sophorose and lactose. In contrast, no cellulases are induced on glycerol and the respective genes are repressed on glucose. We therefore investigated the transcriptome on these five carbon sources in light and darkness and aimed to identify genes specifically expressed under cellulase inducing conditions. These conditions are characterized by a significant enrichment of genes involved in C-compound and carbohydrate degradation and transport among the upregulated gene set. Genes down-regulated under inducing conditions show a significant enrichment in amino acid metabolism and energy metabolism. We were further interested whether light dependent regulation is clustered in the genome and if the carbon source is relevant for activation of light dependent clusters. We found that light dependent clustering predominantly occurs upon growth on cellulose, with the most significant regulation in a gene cluster comprising env1. This cluster appears on glucose as well, but is not down regulated in mutants of blr1 or blr2. Also cbh2, the arabinofuranosidase gene abf2 and the histone acetyltransferase gene gcn5 are part of light dependent clusters. Hierarchical clustering of gene expression patterns was performed to reveal functional divergence of gene regulation with respect to light response or carbon specific regulation. Glycoside hydrolase genes follow the whole transcriptome pattern with carbon source being superior to light in terms of regulation. ENV1 in in part the G-protein beta subunit GNB1 were found to be crucial for carbon source specific regulation of G-protein coupled receptors, genes involved in secretion, sulphur metabolism and oxidative processes as well as transporters. We conclude that clustered regulation of light responsive genes preferentially occurs upon growth cellulose and that ENV1 and to a lesser extent GNB1 play a role in carbon source dependent regulation of specific gene groups in light.

Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay. Chiaki Hori[1,2], Kiyohiko Igarashi[1], David Hibbett[3], Bernard Henrissat[4], Masahiro Samejima[1], Dan Cullen[2]. 1) Graduate School of Agricultural and Life sciences, University of Tokyo, Tokyo, Japan; 2) Forest Products Laboratory, USDA, Madison, WI; 3) Biology Department, Clark University, Worcester, MA; 4) CNRS, Marseille, France.

Many efficient wood decay fungi belong to the Polyporales, and these can be categorized as white-rot fungi or brown-rot fungi, based on decay patterns. White-rot fungi degrade cell wall polysaccharides such as cellulose and hemicellulose as well as the more recalcitrant phenylpropanoid polymer, lignin. In contrast, brown-rot fungi depolymerize the polysaccharides but the modified lignin remains in the wood. Comparative analysis of white- and brown-rot gene repertoires and expression profiles have revealed substantial variation but considerable uncertainty persists with respect to precise mechanisms. Addressing this issue, we performed genome-wide analysis of carbohydrate-active enzymes (CAZy) and some oxidative enzymes related to polysaccharides degradation in eleven white- and brown-rot fungi. This analysis included classifying and enumerating genes from three recently sequenced polyporales *Bjerkandera adusta*, *Ganoderma sp.* and *Phlebia brevispora*. Furthermore, comparative secretomic analysis of seven Polyporales grown on wood culture were conducted. Summarizing, the average number of genes coding CAZy in the genomes of white-rot fungi was 373, significantly more than the 283 observed in brown-rot fungi. Notably, white-rot fungi have genes encoding cellulase and hemicellulase such as those belonging to glycoside hydrolase (GH) families 6, 7, 9 and 74, whereas these are lacking in genomes of brown-rot polyporales. White-rot genes encoding oxidative enzymes potentially related to cellulose degradation such as cellobiose dehydrogenase (CDH), polysaccharides monooxygenase (PMO, formerly GH61), cytochrome b562 with cellulose-binding module, are also increased relative to brown-rot fungi. Indeed, secretomic analysis identified GH6, GH7, CDH and PMO peptides only in white-rot fungi. Overall, these results show that, relative to brown rot fungi, white rot polyporales maintain greater enzymatic diversity supporting lignocellulose attack.

Transcription factor shuttling during cellulase induction in *Trichoderma reesei*. Alex Lichius, Christian P. Kubicek, Verena Seidl-Seiboth. Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria.

For economically feasible production of liquid fuels and other value-added compounds from lignocellulosic plant material, strategies are required to boost cellulolytic and hemicellulolytic enzyme production by industrially relevant fungi. One promising approach is to modulate the transcriptional control mediating release from carbon catabolite repression (CCR) and induction of cellulase, hemicellulase and xylanase gene expression. To better understand the underlying molecular dynamics during induction, we characterized nucleo-cytoplamic shuttling of the two transcription factors carbon catabolite repressor 1 (CRE1) and xylanase regulator 1 (XYR1) of *Trichoderma reesei* by means of live-cell imaging. In submerged cultures, nuclear import and export of CRE1 upon repression and induction, respectively, occurred within minutes and therefore was generally faster than shuttling of XYR1. Under CCR conditions XYR1 expression levels were very low, and its nuclear signal required up to one hour to significantly increase upon replacement into an inducing carbon source. Cultured directly under inducing conditions, nuclear accumulation of XYR1 was detectable after about 20h post inoculation, and strongly increased within the following 24 hours. CRE1 under the same conditions was localized exclusively to the cytoplasm. In plate cultures, nuclear recruitment of CRE1 and XYR1 differed within the central area, the subperiphery and the periphery of the colony depending on the provided carbon source. Most interestingly, under inducing conditions we found evidence for increased nuclear recruitment of CRE1 in the central area, correlating with strong nuclear import of XYR1 in the same region. Notably, the cytoplasmic signal of CRE1 was usually elevated in leading hyphae, whereas XYR1 was never significantly recruited to the colony periphery. Taken together our data provide the first temporal resolution of transcription factor shuttling during the induction of cellulase gene expression in *Trichoderma reesei*, and reveal some interesting differences between the subcellular localization of CRE1 and XYR1 in submerged and plate cultures, respectively. These differences indicate that the mycelial organization during fungal growth might be another important regulatory element to consider for the industrial scale production of cellulolytic enzymes.

Friday, March 15 3:00 PM–6:00 PM
Merrill Hall

Pathogenic Signaling via Effector Proteins

Co-chairs: Brett Tyler and Sebastien Duplessis

Dissecting nuclear immunity using Arabidopsis downy mildew effector as probes. Marie-Cecile Caillaud[1], Lennart Wirthmueller[1,2], Shuta Asai[1], Sophie Piquerez[1], Georgina Fabro[1,3], Jonathan Jones[1]. 1) The Sainsbury Laboratory, Norwich, United Kingdom; 2) John Innes Centre, Norwich, United Kingdom; 3) Present address: CIQUIBIC-CONICET, Universidad Nacional de Cordoba, Argentina.

An important role in plant defence has been attributed to nuclear dynamics, since a growing number of reports reveal that the nuclear localization of key components of plant immunity is essential for disease resistance. Recent studies suggest that effectors may manipulate host transcription or other nuclear process for the benefit of the pathogen. However, the specific mechanisms by which these effectors promote susceptibility remain unclear. The interaction between Arabidopsis and *Hyaloperonospora arabidopsidis* (*Hpa*) has been studied intensively during the past twenty years, and it has become one of the most well-understood model systems to help us understand pathogen effector biology and the plant immune system. The recent identification of 15 nuclear-localized *Hpa* effectors (HaRxLs) provides a powerful tool to dissect plant nuclear immunity. When stably expressed *in planta*, nuclear-HaRxLs cause diverse developmental phenotypes which highlight their interferences with fundamental plant regulatory mechanisms. Remarkably, nuclear HaRxLs-plant targets are often transcriptional regulators, which may act in complex with immunity co-factors. Here, we report recent insights into our understanding of the arms race between obligate pathogen and its host.

The mutualistic fungus *Laccaria bicolor* uses the effector protein MiSSP7 to alter host jasmonate signaling and establish symbiosis. Claire Veneault-Fourrey[1], Jonathan Plett[1,3], Yohann Daguerre[1], Aurélie Deveau[1], Annegret Kohler[1], Jennifer Morrell-Falvey[2], Annick Brun[1], Francis Martin[1]. 1) UMR1136 IaM_INRA/UHP, Lorraine Univ / INRA, Lab of Excellence ARBRE, Nancy, France; 2) Oak Ridge National Laboratory, Oak Ridge, TN 37831-6422, USA; 3) Hawkesbury Institute for the Environment, University of Western Sydney, Australia.

Roots of most trees form a nutrient-acquiring symbiosis with mutualistic fungi. Mycorrhiza-induced Smal Secreted protein MiSSP7, a fungal effector protein necessary for the mutualistic interaction between of the ectomycorrhizal fungus *Laccaria bicolor* and *Populus* spp. host trees, is secreted by the fungus in contact with plant tissues and is taken up via endocytosis into plant cells where it localizes to the nucleus and targets plant transcription through an unknown mechanism Here we demonstrate that MiSSP7 interacts with the jasmonic acid receptorJAZ6 of *Populus trichocarpa* and that PtJAZ6 interacts with a number of other nuclear localized proteins that likely form a DNA binding complex. MiSSP7 is able to block jasmonic acid signaling in both L. bicolor host and non-host plants, likely through its interaction with a jasmonate receptor. Loss of MiSSP7 expression in L. bicolor can be complemented by transgenically varying the transcription of PtJAZ6 or through inhibiting jasmonic acid biosynthesis in poplar roots. We conclude that MiSSP7, in contrast to arbuscular mycorrhizal fungi and pathogenic bacteria that promote jasmonate signaling to colonize host tissues, is a novel effector used to promote mutualism by blocking jasmonic acid signaling. In addition to MiSSP7, *L. bicolor* expresses other MiSSPs to communicate with its host-plant. In particular, we demonstrate that MiSSP8 an apoplastic effector is required for symbiosis.

Plett JM, et al. (2011) Curr Biol . 21:1197-1203.

Identification and characterization of an RXLR-like effector family from medically relevant fungi. Shiv D. Kale[1*], Kelly C. Drews[1,2], Helen R. Clark[1,3], Hua Wise[1,4], Vincenzo Antignani[1], Tristan A. Hayes[1,2], Christopher B. Lawrence[1,2], Brett M. Tyler[4,5]. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 5) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Fungal infections have become an increasingly significant problem for immunocompromised individuals, transplant recipients, the elderly, several cases involving healthy individuals. There is a significant growth in incidences of morbidity and mortality associated with medically important fungi, specifically Aspergillus species. Aspergillus fumigatus virulence has been attributed to production of pigments, adhesins on the surface of the cell wall, secreted proteases, and mycotoxins. Current treatments consist of oral corticosteroids, antifungal medications, and/or surgery to remove aspergillomas. Many of these treatments have substantial shortcomings. Detection and diagnosis is also weighty problem as most clinical tests take weeks for results allowing the infection to proceed. Appropriately, the paradigm for human fungal interactions has been focused on the host deficiencies mediating virulence of opportunistic pathogenic fungi. There has been substantial progress in identifying and characterizing secreted proteins (effectors) from bacterial, oomycete, and fungal plant pathogens. A subset of these effector proteins are able to enter host cells and modulate host intracellular functions. Using our bioinformatics pipeline we have been able to identify a family of secreted proteins from A. fumigatus sharing a conserved N-terminal RXLR-like motif. We found this family is expanded amongst primary fungal pathogens. The RXLR and RXLR-like motifs from known intracellular effectors of plant pathogenic and mutualistic oomycetes and fungi have been shown to facilitate effector entry into plant cells via binding external phosphatidylinositol-3-phosphate (PI3P). Here we describe AF2, a candidate effector from A. fumigatus that contains a N-terminal RxLR-like motif. Through the use of confocal microscopy and flow cytometry we show AF2 is rapidly able to enter several primary and immortalized mammalian cell lines. Through the use of isothermal titration calorimetry and liposome binding assays we show AF2 has nanomolar binding affinity for PI3P, and does not bind other mono or poly-PIPs that we have tested thus far. Based on our bioinformatics and biochemical analysis we postulate AF2 is a secreted effector protein capable of rapidly translocating into mammalian cells. We will present our latest findings on the physiological relevance of AF2.

Identification and functional assay of Phytophthora sojae avirulence effectors. Yuanchao Wang, Suomeng Dong, Weixiao Yin. Plant Pathology Dept, Nanjing Agri Univ, Nanjing, China.

Phytophthora sojae is a notorious oomycete pathogen producing a great loss on global soybean production annually. The disease outcome between soybean and P. sojae depends on whether hosts could recognize pathogen avirulence effectors. Recently identified oomycete avirulence effectors are characterized by N-terminal host entry motif (RxLR motif), sequence and transcriptional polymorphisms between virulent and avirulent strains. Benefit from 454 genome sequencing and solexa transcriptome sequencing of P. sojae strains, eight RxLR effectors are bioinformatically identified, genetic mapping suggested that two of them perfectly matched Avr3b and Avr1d phenotype respectively. Transient expression of the ORF from avirulence strain on soybean specifically triggered Rps3b and Rps1d mediated program cell death, respectively. confirming that they encodes avirulence effector Avr3b and Avr1d. Transient expression of Avr3b and Avr1d on Nicotiana benthamiana could promote the infection of Phytophthora capasici, suggesting both avirulence effectors could suppress plant immunity and contribute to pathogen infection. Silencing of Avr3b impaired the virulence of Phytophthora sojae. Our progress in elucidating the mechanism under the inhibiting plant immunity by these effectors will be presented.

Fungal lipoxygenases: a novel instigator of asthma? Gregory J. Fischer[1], Katharyn Affeldt[3], Erwin Berthier[2], Nancy P. Keller[1,2,3]. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Statement of Purpose: Fungi have long been associated with asthmatic diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown. We propose that fungal lipoxygenase enzymes and their eicosanoid products are involved in asthmatic diseases. Human 5-lipoxygenase derived leukotrienes induce inflammation, mucus secretion, vasodilation, and bronchial constriction. We hypothesize that the fungal pathogen *Aspergillus fumigatus* is capable of secreting a 5-lipoxygenase homolog, LoxB, that participates in eicosanoid production, including leukotrienes. This secreted homolog is translocated into lung epithelial cells, participates in the production of leukotriene and other eicosanoids, and exacerbates asthmatic responses, such as bronchoconstriction. Together, this work will help delineate the role fungal products play in asthmatic diseases. **Methods:** We are assessing fungal interactions with lung epithelial cells using a microfluidic *in-vitro* platform followed by murine asthma model research. To assess the effects of LoxB overexpression, mass spectrometry was used to identify eicosanoid oxylipins within culture supernatants. **Results:** We have identified an *Aspergillus fumigatus* lipoxygenase, LoxB, with high identity to human 5-lipoxygenase. Moreover, we have identified a motif in LoxB that may mediate entry into lung epithelial cells. To fully understand the impact of LoxB in asthma, we have developed an *Aspergillus fumigatus* strain that overexpresses LoxB. Overexpression of LoxB results in increased levels of various eicosanoids that are known to cause airway hyperresponsiveness and increased mucus production. Future work will focus on characterizing the effect these eicosanoid products have on the airway and whether fungal effector translocation result in increased leukotriene levels.

***Magnaporthe oryzae* has evolved two distinct mechanisms of effector secretion for biotrophic invasion of rice.** Martha C. Giraldo[1], Yasin F. Dagdas[2], Yogesh K. Gupta[2], Thomas A. Mentlak[2,4], Mihwa Yi[1], Hiromasa Saitoh[3], Ryohei Terauchi[3], Nicholas J. Talbot[2], Barbara Valent[1]. 1) Plant Pathology, Kansas State University, Manhattan, KS. USA; 2) School of Biosciences, University of Exeter, EX4 4QD, UK; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003 Japan; 4) Cambridge Consultants Ltd, Cambridge, CB4 0DW, U.K.

Pathogens secrete effector proteins into host tissue to suppress immunity and cause disease. Pathogenic bacteria have evolved several distinct secretion systems to target specific effector proteins during pathogenesis, but it was not previously known if fungal pathogens require different secretory mechanisms. We present evidence that the blast fungus *Magnaporthe oryzae* possesses distinct secretion systems for delivering effector proteins during biotrophic invasion of rice cells. *M. oryzae* secretes cytoplasmic effectors targeted for delivery inside rice cells and apoplastic effectors targeted to the extracellular space. Cytoplasmic effectors preferentially accumulate in the biotrophic interfacial complex (BIC), a novel in planta structure located beside the tip of the initially filamentous invasive hypha and then remaining next to the first differentiated bulbous invasive hypha cell. In contrast, apoplastic effectors remain in the extracellular compartment uniformly surrounding the invasive hypha inside the invaded cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A (BFA) treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors. Fluorescence Recovery After Photobleaching experiments confirmed that cytoplasmic effectors continued to accumulate in BICs in the presence of BFA. Analysis of mutants showed that the BIC is associated with a novel form of secretion involving exocyst components, Exo70 and Sec5, and the t-SNARE Sso1, which are required for efficient delivery of effectors into plant cells and are critical for pathogenicity. By contrast, effectors which function between the fungal cell wall and plant plasma membrane are secreted from invasive hyphae to the apoplast by the ER-Golgi secretory pathway conserved in eukaryotes. We propose a model for the distinct secretion systems that the rice blast fungus has evolved to achieve tissue invasion.

Domains for plant uptake of Ustilago maydis secreted effectors. Anupama Ghosh, Armin Djamei, Shigeyuki Tanaka, Regine Kahmann. Max PIanck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-Von-Frisch-Strasse 10, D-35043 Marburg, Germany.

The genome of the corn smut fungus Ustilago maydis codes for a large repertoire of secreted effectors. Some of them play crucial roles for virulence and establishment of the biotrophic phase. The chorismate mutase Cmu1 is one such secreted translocated effector of U. maydis. cmu1 deletion strains are attenuated in virulence that is attributed to higher salicylate levels in plants infected with the mutant strain, most likely through alterations in the channeling of chorismate from the plastids to the cytosol. Here we identify the motif in Cmu1 that is necessary for the translocation of the protein across the plant plasma membrane and present a mutational analysis of this region. To test for uptake we assayed the ability of mutant proteins to complement a cmu1 mutant strain as well as the retained ability to complement the growth defect of a Daro7 strain of S. cerevisiae in minimal medium. By deletion analysis a region of 20 amino acids adjacent to the signal peptide was shown to be essential for the translocation. Microscopic analysis of maize tissue infected with U. maydis strains expressing Cmu1-mcherry fusion proteins with or without the probable uptake motif revealed that the 20 amino acid motif allows binding of the protein to an as yet unknown plant plasma membrane component. We hypothesize that the translocation of Cmu1 across the plant plasma membrane is a two step process; initiated by binding followed by translocation across the membrane. In addition, we present results where the 20 amino acid motif is substituted by motifs from other effectors.

Penetration-specific effectors from *Phytophthora parasitica* favour plant infection. Edouard Evangelisti[1*], Benjamin Govetto[2], Naima Minet-Kebdani[1], Marie-Line Kuhn[1], Agnes Attard[1], Franck Panabieres[1], Mathieu Gourgues[1]. 1) UMR Institut Sophia Agrobiotech, INRA/CNRS/Université de Nice, Sophia Antipolis, France; 2) Institut Méditerranéen de Biodiversité et d'Écologie marine et continentale (IMBE), CNRS-INEE - IRD -Aix Marseille Université - Université d'Avignon - Institut Pytheas.

Oomycetes are major crop pests which cause million dollars losses every year. To date only a few efficient chemicals are available against these filamentous microorganisms. A better understanding of the molecular events occuring during plant-oomycete interactions will help to propose new strategies for crop protection. We performed a transcriptional analysis in order to identify oomycete penetration-specific genes and identified a set of penetration-specific effectors (PSE) bearing a RXLR motif. This motif was previously shown to promote effector import into plant cells during the biotrophic stage in feeding structures called haustoria. Here we report the functional analysis of three candidate genes, referred to as PSE1, PSE2 and PSE3. The three effectors were able to abolish plant defense responses when transiently expressed in Nicotiana plants. Moreover, constitutive expression of PSE1 and PSE3 in *A. thaliana* led to an enhanced susceptibility to *P. parasitica* infection suggesting a role for these proteins in *P. parasitica* pathogenicity. Transgenic Arabidopsis lines accumulating PSE1 protein showed several developmental perturbations that were associated with altered auxin physiology. Root growth inhibition assays showed that auxin signaling pathway is not altered by PSE1 accumulation. Nevertheless, the coiled-root phenotype and the enhanced susceptibility of PSE1-expressing lines to *P. parasitica* were reverted by synthetic auxin 2,4-D supply, or treatment with the auxin efflux inhibitor TIBA suggesting that a reduced auxin accumulation is responsible for these phenotypes. This hypothesis was confirmed by a reduced activity of the pDR5 auxin sensitive promoter at the root apex. The alteration of the expression pattern observed for two auxin efflux carriers, PIN4 and PIN7 suggests that a perturbation of auxin efflux could be responsible for the PSE1 associated defects. We proposed that PSE1 could favour *P. parasitica* virulence by interfering with auxin content. Our results show that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.

Friday, March 15 3:00 PM–6:00 PM
Chapel

Cell Wall, Polarity and Hyphal Tip Growth

Co-chairs: Stephan Seiler and Ernestina Castro-Longoria

The function of Rho type small GTPases for cell polarity in *Ustilago maydis*. Britta Tillmann[1], Michaela Wehr[1], Sonja Frieser[1], Kay Oliver Schink[2], Johannes Freitag[1], Michael Bölker[1]. 1) Dept Biol, Univ Marburg, Marburg, Germany; 2) Institute for Cancer Research, the Norwegian Radium Hospital, Oslo University Hospital, Montebello, 0310 Oslo, Norway.

Establishment of cell polarization requires the coordinated transport and localized fusion of secretory vesicles. This process is controlled by Rho-type GTPases that act as molecular switches. Temporal and spatial activation of Rho-GTPases depends on specific guanine nucleotide exchange factors (GEFs). Inactivation of Rho proteins is achieved via interaction with GTPase activating proteins (GAPs) that stimulate the low intrinsic GTPase activity of Rho proteins. During its life cycle, *U. maydis* switches between budding and filamentous growth. The Rho type GTPase Rac1 is the main regulator of this morphogenic transition. The highly related Cdc42 is required for cell separation after mitosis and for formation of retraction septa during filamentous growth. We could show that the activator of Rac1, the Rho-GEF Cdc24 is subject to a negative autoregulatory feedback loop. Active Rac1 triggers Cla4 dependent multisite phosphorylation of a C-terminal destruction box. This results in rapid degradation of Cdc24 and release of a ternary complex containing active Rac1, the scaffold protein Bem1 and the Rac1 effector kinase Cla4. The active Rac1 is subsequently inactivated by GAPs that localize in a ring-like fashion underneath of the tip. Both destruction of Cdc24 and inactivation of Rac1 serve to delimit Rac1 activity to the very tip of the fungal hypha. Sustained polarized growth is further supported by recycling of inactive Rac1 to the hyphal tip. This is achieved either by interaction with the Rho protein GDP dissociation inhibitor Gdi1 or via endocytosis. Active Rho-GTPases recruit specific effectors that trigger the localized fusion of secretory vesicles at the hyphal tip. We found that both Cdc42 and Rac1 interact with Sec3, a subunit of the multiprotein exocyst complex. We have identified several homologs of exocyst subunits in *U. maydis* and tested them for functions during polar growth. We could demonstrate that Rac1 is critical for proper localization of the exocyst landmark protein Sec3. We have identified the *U. maydis* homolog of Smg-GDS, an unconventional activator of Rho GTPases in mammals. Smg-GDS contains a number of armadillo repeats and interacts with both Cdc42 and Rac1. Deletion of the Smg-GDS gene reduces significantly mating and filament formation, indicating that it contributes to regulation of cell polarity.

A quantitative model of hyphal tip growth based on the spatial distribution of exocyst subunits in the human fungal pathogen *Candida albicans*. David Caballero- Lima, Ilyana Kaneva, Simon Watton, C. Jeremy Craven, Peter Sudbery. Dept Molecular Biol & Biotech, Sheffield Univ, Sheffield, S Yorkshire, United Kingdom.

We present a quantitative three dimensional treatment of fungal hyphal growth which adapts previous theoretical treatments in the light of advances in our knowledge of the components of polarised growth and their location as revealed by GFP fusions. The model is based on the proposition that vesicles fuse with the hyphal tip at a rate determined by the experimentally observable local density of exocyst components. Enzymes such as b-1,3 glucan synthase are embedded in the plasma membrane by this process and continue to synthesize cell wall until they are removed from the membrane by endocytosis. The time development of the spatial distribution of the synthase molecules arises from the model. We test the model in the hyphae of the human fungal pathogen *Candida albicans* by quantitative measurements of the distribution of exocyst components and membrane components such as GFP-Rho1, the regulatory subunit of b1,3 glucan synthase, Rom2-GFP, the GEF for Rho1, and the location of actin cortical patches. We show that the predicted shape and width of the hyphae are in good agreement with that predicted by the model, provided that endocytosis acts to remove cell wall synthesizing enzymes at the subapical band of cortical actin patches. Thus the pattern of tip growth of fungal hyphae can be satisfactorily explained by a simple but quantitative model rooted within the known molecular processes of polarized growth. At the same time the model exposes the areas of uncertainty which need to be addressed by future experimentation.

Cell wall integrity signaling in *Aspergillus fumigatus*. Johannes Wagener, Karl Dichtl, Christoph Helmschrott, Sweta Samantaray, Franziska Dirr, Michael Neubauer. Max von Pettenkofer-Institut, University of Munich, Munich, Germany.

Aspergillus fumigatus is an opportunistic pathogen and the most frequent cause of a severe invasive infection termed invasive aspergillosis. Similar to other fungi, this mold is surrounded by a robust cell wall that defines its shape and protects it from physical stress. We have characterized the cell wall integrity (CWI) pathway of *A. fumigates*. It comprises at least three major membrane anchored cell wall stress sensors with partially overlapping functions (Wsc1, Wsc3 and MidA), the guanine nucleotide exchange factor Rom2, a Rho GTPase, protein kinase C and a MAP kinase signaling module. We have shown that the principal CWI components are well conserved from yeasts to filamentous fungi. Though, the importance of the individual components for the fungal physiology, e.g., cell polarity and conidiation, may significantly differ. Our data stress the importance of the CWI pathway for the antifungal drug susceptibility and virulence of this pathogen.

Optimization of polarity establishment through coupling of multiple feedback loops. Roland Wedlich-Soldner[1], Tina Fresisinger[1], Ben Kluender[2], Nikola Mueller[1], Gisela Beck[1], Garwin Pichler[4], Jared Johnson[3], Richard Cerione[3], Erwin Frey[2]. 1) Cellular Dynamics adn Cell Patterning, Max Planck Institute of Biochemistry, Martinsried, Germany; 2) Arnold Sommerfeld Center for Theoretical Physics, Ludwig Maximilians University Munich, Munich, Germany; 3) Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, NY, USA; 4) Department of Biology II, Ludwig Maximilians University Munich, Martinsried, Germany.

Establishment of cell polarity - or symmetry breaking - relies on local accumulation of polarity regulators. While simple positive feedback is sufficient to drive symmetry breaking, it is highly sensitive to stochastic fluctuations typical for living cells. By integrating mathematical modeling with quantitative experimental validations we now show that in the yeast Saccharomyces cerevisiae only a combination of actin- and Guanine nucleotide Dissociation Inhibitor (GDI)-dependent recycling of the central polarity regulator Cdc42 is capable of establishing robust cell polarity at a single site during yeast budding. The GDI pathway consistently generates a single polarization site, but requires Cdc42 to cycle rapidly between its active and inactive form, and is therefore highly sensitive to perturbations of the GTPase cycle. Conversely, actin-mediated recycling of Cdc42 induces robust symmetry breaking but

cannot restrict polarization to a single site. Our results demonstrate how cells optimize symmetry-breaking through coupling between multiple feedback loops.

Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis. Vincent Bulone. Sch Biotech, Royal Inst Biotech (KTH), Stockholm, Sweden.

Cell wall polysaccharides play a central role in vital processes like the morphogenesis and growth of eukaryotic micro-organisms. Thus, the enzymes responsible for their biosynthesis represent potential targets of drugs that can be used to control diseases provoked by pathogenic species. One of the most important features that distinguish oomycetes from true fungi is their specific cell wall composition. The cell wall of oomycetes essentially consists of (1®3)-b-glucans, (1®6)-b-glucans and cellulose whereas chitin, a key cell wall component of fungi, occurs in minute amounts in the walls of some oomycete species only. Thus, the cell walls of oomycetes share structural features with both plants [cellulose; (1®3)-b-glucans] and true fungi [(1®3)-b-glucans, (1®6)-b-glucans and chitin in some cases]. However, as opposed to the fungal and plant carbohydrate synthases, the oomycete enzymes exhibit specific domain compositions that may reflect polyfunctionality. In addition to summarizing the major structural differences between oomycete and fungal cell walls, this presentation will compare the specific properties of the oomycete carbohydrate synthases with the properties of their fungal and plant counterparts, with particular emphasis on chitin, cellulose and (1®3)-b-glucan synthases. The significance of the association of these carbohydrate synthases with membrane microdomains similar to lipid rafts in animal cells will be discussed. In addition, distinguishing structural features within the oomycete class will be highlighted with the description of our recent classification of oomycete cell walls in three different major types. Genomic and proteomic analyses of selected oomycete and fungal species will be correlated with their cell wall structural features and the corresponding biosynthetic pathways.

Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function. Lakshmi Preethi Yerra, Steven Harris. University of Nebraska-Lincoln, Lincoln, NE.

In the filamentous fungus Aspergillus nidulans, the transition from hyphal growth to asexual development is associated with dramatic changes in patterns of cellular morphogenesis and division. These changes enable the formation of airborne conidiophores that culminate in chains of spores generated by repeated budding of phialides. Our objective is to characterize the regulatory modules that mediate these changes and to determine how they are integrated with the well-characterized network of transcription factors that regulate conidiation in A. nidulans. Because protein phosphorylation is likely to be a key component of these regulatory modules, we have exploited the availability of A. nidulans post-genomic resources to investigate the roles of protein kinases and phosphatases in developmental morphogenesis. We have used the protein kinase and phosphatase deletion mutant libraries made available by the Fungal Genetics Stock Center to systematically screen for defects in conidiophore morphology and division patterns. Our initial results implicate ANID_11101.1 (=yeast Hsl1/Gin4) in phialide morphogenesis, and also reveal the importance of ANID_07104.1 (=yeast Yak1) in the maintenance of cell integrity during asexual development. Additional deletion mutants with reproducible defects have been identified and will be described in detail. We will also summarize initial results from double mutant analyses that attempt to place specific protein kinase deletions within the regulatory network that controls conidiation.

Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites. Diego Delgado-Álvarez[1], S. Seiler[2], S. Bartnicki-García[1], R. Mouriño-Pérez[1]. 1) CICESE, Ensenada, Mexico; 2) Georg August University, Göttingen, Germany.

The machinery responsible for cytokinesis and septum formation is well conserved among eukaryotes. Its main components are actin and myosins, which form a contractile actomyosin ring (CAR). The constriction of the CAR is coupled to the centripetal growth of plasma membrane and deposition of cell wall. In filamentous fungi, such as Neurospora crassa, cytokinesis in vegetative hyphae is incomplete and results in the formation of a centrally perforated septum. We have followed the molecular events that precede formation of septa and constructed a timeline that shows that a tangle of actin filaments is the first element to conspicuously localize at future septation sites. We named this structure the SAT for septal actin tangle. SAT formation seems to be the first event in CAR formation and precedes the recruitment of the anillin Bud-4, and the formin Bni-1, known to be essential for septum formation. During the transition from SAT to CAR, tropomyosin is recruited to the actin cables. . Constriction of the CAR occurs simultaneously with membrane internalization and synthesis of the septal cell wall.

Visualization of apical membrane domains in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM). Norio Takeshita[1], Yuji Ishitsuka[2], Yiming Li[2], Ulrich Nienhaus[2], Reinhard Fischer[1]. 1) Dept. of Microbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Institute for Applied Physics, Karlsruhe Institute of Technology.

Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The size of SRDs is around a few mm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs were analyzed by super-resolution microscope technique, Photoactivated Localization Microscopy (PALM). Membrane domains were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. Size, number, distribution and dynamics of membrane domains, and dynamics of single molecules were investigated. Time-laps analysis revealed the dynamic behavior of exocytosis.

Friday, March 15 3:00 PM–6:00 PM
Heather

Sexual Regulation and Evolution in the Fungi

Co-chairs: Frances Trail and Nicolas Corradi

Clonality and sex impact aflatoxigenicity in *Aspergillus* populations. Ignazio Carbone[1], Bruce W. Horn[2], Rodrigo A. Olarte[1], Geromy G. Moore[3], Carolyn J. Worthington[1], James T. Monacell[4,1], Rakhi Singh[1], Eric A. Stone[5,4], Kerstin Hell[6], Sofia N. Chulze[7], German Barros[7], Graeme Wright[8], Manjunath K. Naik[9]. 1) Department of Plant Pathology, NC State University, Raleigh, NC, USA; 2) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA, USA; 3) Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, LA, USA; 4) Bioinformatics Research Center, NC State University, Raleigh, NC, USA; 5) Department of Genetics, NC State University, Raleigh, NC, USA; 6) International Institute of Tropical Agriculture, Cotonou, Republic of Benin; 7) Departamento de Microbiologia e Inmunologia, Universidad Nacional de Rio Cuarto, Cordoba, Argentina; 8) Department of Primary Industries, Queensland, Kingaroy, Australia; 9) Department of Plant Pathology, College of Agriculture, Karnataka, India.

Species in *Aspergillus* section *Flavi* commonly infect agricultural staples such as corn, peanuts, cottonseed, and tree nuts and produce an array of mycotoxins, the most potent of which are aflatoxins. *Aspergillus flavus* is the dominant aflatoxin-producing species in the majority of crops. Populations of aflatoxin-producing fungi may shift in response to: (1) clonal amplification that results from strong directional selection acting on a nontoxin- or toxin-producing trait; (2) disruptive selection that maintains a balance of extreme toxigenicities and diverse mycotoxin profiles; (3) sexual reproduction that results in continuous distributions of toxigenicity; or (4) female fertility/sterility that impacts the frequency of sexual reproduction. Population shifts that result in changes in ploidy or nuclear DNA composition (homokaryon versus heterokaryon) may have immediate effects on fitness and the rate of adaptation in subsequent fungal generations. We found that *A. flavus* populations with regular rounds of sexual reproduction maintain higher aflatoxin concentrations than predominantly clonal populations and that the frequency of mating-type genes is directly correlated with the magnitude of recombination in the aflatoxin gene cluster. Genetic exchange within the aflatoxin gene cluster occurs via crossing over between divergent lineages in populations and between closely related species. During adaptation, specific toxin genotypes may be favored and swept to fixation or be subjected to drift and frequency-dependent selection in nature. Results from mating experiments in the laboratory indicate that fertility differences among lineages may be driving genetic and functional diversity. Differences in fertility may be the result of female sterility, changes in heterokaryotic state, DNA methylation, or other epigenetic modifications. The extent to which these processes influence aflatoxigenesis is largely unknown, but is critical to understand for both fundamental and practical applications, such as biological control. Our work shows that a combination of population genetic processes, especially asexual/sexual reproduction and fertility differences coupled with ecological factors, may influence aflatoxigenicity in these agriculturally important fungi.

Toolkit for sexual reproduction in the genome of Glomus spp; a supposedly ancient asexual lineage. Nicolas Corradi. Department of Biology, University of Ottawa, Ottawa, Ontario, Canada.

Arbuscular mycorrhizal fungi (AMF) are involved in a critical symbiosis with the roots of most land plants;the mycorrhizal symbiosis. Despite their importance for terrestrial ecosystems worldwide, many aspects of AMF evolution and genetics are still poorly understood, resulting in notorious scientific frustrations and intense debates; especially regarding the genetic structure of their nuclei (heterokaryosis vs homokayosis) and their mode of propagation (long-term clonality vs cryptic sexuality). This will aim address the latter aspect of their biology - i.e. their mode of reproduction - by cataloguing and highlighting emerging evidence, based on available genome sequence data, for the presence of a cryptic sexual cycle in the AMF . In particular, investigations along available genome and transcriptome data from several AMF species have unravelled the presence of a battery of genes that are commonly linked with sexually-related processes in other fungal phyla. These include a gene-set required for the initiation and completion of a conventional meiosis, as well as many other genomic regions that are otherwise found to play a pivotal role in fungal partner recognition. The origin, diversity and functional analysis of some of these sexually-related genes in AMF will be discussed.

Comparative transcriptomics identifies new genes for perithecium development. Frances Trail[1], Usha Sikhakolli[1], Kayla Fellows[1], Nina Lehr[2], Jeffrey Townsend[2]. 1) Department of Plant Biology, Michigan State Univ, East Lansing, MI; 2) Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT.

In recent years, a plethora of genomic sequences have been released for fungal species, accompanied by functional predictions for genes based on protein sequence comparisons. However, identification of genes involved in particular processes has been extremely slow, and new methodologies for identifying genes involved in a particular process have not kept pace with the exponential increase in genome sequence availability. We have performed transcriptional profiling of five species of Neurospora and Fusarium during six stages of perithecium development. We estimated the ancestral transcriptional shifts during the developmental process among the species and identified genes whose transcription had substantially and significantly shifted during the evolutionary process. We then examined phenotypes of knockouts of genes whose expression greatly increased in *Fusarium graminearum* perithecium development. In numerous cases, gene disruption resulted in substantial changes in perithecium. These genes were not previously identified as candidates for function in perithecium development, illustrating the utility of this method for identification of genes associated with specific functional processes.

Rapid evolution of female-biased genes: a novel example from the eukaryotic model organism *Neurospora crassa*. Hanna Johannesson, Carrie Whittle. Evolutionary Biology, Uppsala University, Uppsala, Sweden.

In animals and plants, sex-biased gene expression plays a major role in gene evolution. In particular, reproductive genes with male-biased expression tend to exhibit rapid protein evolution and reduced codon bias as compared to female-biased or unbiased genes. Minimal data are available for fungi. Here, we demonstrate that sex-biased expression is associated with gene evolution in the filamentous fungus *Neurospora crassa*, but in contrast to animals and plants, the rapid evolution occurs for female-biased genes. Based on analyses of >25,000 expressed sequence tags (ESTs) from male (conidial), female (protoperithecial) and vegetative (mycelial) tissues, we show that reproductive genes with female-biased expression exhibit faster protein evolution and reduced optimal codon usage than male-biased genes and vegetative genes. Furthermore, our data suggest that female-biased genes are also more apt to experience selective sweeps. The sex-biased expression effects are observable at the species and population level. We argue that the rapid molecular evolution of female-biased genes is best explained by sexual selection via female-female competition, but could also result from mate-choice and/or directional natural selection.

Self-attraction can not bypass the requirement for two mating type genes during sexual reproduction in *Neurospora crassa*. Katherine A. Borkovich, Hyojeong Kim, Sara Wright, Gyungsoon Park, Shouqiang Ouyang, Svetlana Krystofova. Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

The pheromone receptor PRE-2 is highly expressed in male and female reproductive structures of *mat a* strains in *Neurospora crassa*. Trichogynes from D*pre-2 mat a* protoperithecia do not respond chemotropically to *mat A* conidia or form mature fruiting bodies or meiotic progeny. Strains with swapped identity due to heterologous expression of *pre-2* or its cognate pheromone *ccg-4* behave normally in crosses with opposite mating-type. Coexpression of *pre-2* and *ccg-4* in the *mat A* background leads to self-attraction and development of barren perithecia that lack ascospores. Further perithecial development is achieved by inactivation of *Sad-1*, a gene required for meiotic gene silencing in *N. crassa*. Results from studies using forced heterokaryons of opposite mating-type strains show that the presence of one receptor and its compatible pheromone is necessary and sufficient for perithecial development and ascospore production. Taken together, the results demonstrate that although receptors and pheromones control sexual identity, the mating-type genes (*mat A* and *mat* a) must be in two different nuclei to allow meiosis and sexual sporulation to occur in *N. crassa*.

Fertility in *Aspergillus fumigatus* and the identification of an additional 'supermater' pair. Céline M. O'Gorman[1], Sameira S. Swilaiman[1], Janyce A. Sugui[2], Kyung J. Kwon-Chung[2], Paul S. Dyer[1]. 1) School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; 2) Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

Aspergillus fumigatus is an opportunistic human pathogen that causes a range of allergic and invasive diseases in severely immunocompromised individuals, with a very high mortality rate typically in excess of 50%. A functional sexual cycle was discovered in 2009 and a highly fertile 'supermater' pair, AFB62 and AfIR928, was later identified from a collection of 50 isolates. Here we describe the results of a larger, worldwide fertility screen and present an additional 'supermater' pair. A set of 126 clinical and environmental *A. fumigatus* isolates were crossed against two Irish reference strains of each mating type. A subset of the eight most-fertile strains was then tested in all pairwise combinations. The pairing of isolates 47-169 x 47-154 had consistently high mating efficiency and outcrossing ability after four weeks, therefore it was chosen as an additional 'supermater' pair for community use in mating projects. It is important to have alternative tester strains to allow for unexpected mating differences when crossing isolates of diverse genetic origins. This is because factors such as heterokaryon incompatibility (*het*) loci and single nucleotide polymorphisms, can considerably influence sexual compatibility. The worldwide fertility screen found that approximately 85% of isolates are sexually fertile, indicating that sexual reproduction should be possible in nature when suitable environments are present. Next, the plasticity of sexual crossing conditions was tested, to determine whether they could be manipulated to increase fertility in crosses involving low-fertility strains of interest. A range of environmental and growth conditions were examined, including incubation temperature, CO_2 level, and oatmeal agar type. Fertility levels were significantly affected by certain parameters. Work is ongoing to integrate these factors to further optimize fertility in the 'supermater' pairs.

Sexual reproduction and mating type function in the penicillin producing fungus *Penicillium chrysogenum*. Julia Böhm[1], Birgit Hoff[1], Simon Wolfers[1], Céline O'Gorman[2], Paul Dyer[2], Stefanie Pöggeler[3], Ulrich Kück[1]. 1) Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Deutschland; 2) School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K; 3) Abteilung Genetik eukaryotischer Mikroorganismen, Institut für Mikrobiologie und Genetik, Georg-August Universität Göttingen, 37077 Göttingen, Deutschland.

Penicillium chrysogenum is a filamentous fungus of major medical and historical importance, being the original and present day industrial source of the antibiotic penicillin with a world market value of about 600 million € per year. The species has been considered asexual for over 100 years and despite concerted efforts it has not been possible to induce sexual reproduction. However, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Utilising knowledge of mating-type (*MAT*) gene organization we now describe conditions under which a sexual cycle can be induced leading to the production of cleistothecia and meiotic ascospores, which were similar to those described recently for *Eupenicillium crustaceum* [2]. Evidence of recombination was obtained using both molecular and phenotypic markers. The newly identified heterothallic sexual cycle was used for strain development purposes, generating offspring with novel combinations of traits relevant to penicillin production.

Furthermore, the *MAT1-1-1* mating-type gene, known primarily for a role in governing sexual identity, was also found to control transcription of a wide range of genes including those regulating penicillin production, hyphal morphology and conidial formation, all traits of biotechnological relevance. For functional characterization *MAT1-1-1* knockout and overexpression strains were generated and analyzed. These discoveries of a sexual cycle and *MAT* gene function are likely to be of broad relevance for manipulation of other asexual fungi of economic importance.

[1] Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, Fleming`s *Penicillium* strain discloses the secret of its sex. Eukaryot Cell 7: 465-470

[2] Pöggeler S, O'Gorman CM, Hoff B, Kück U (2011) Molecular organization of the mating-type loci in the homothallic ascomycete *Eupenicillium crustaceum*. Fungal Biol. 115: 615-624.

The *Sclerotinia sclerotiorum* mating type locus (*MAT*) contains a 3.6-kb region that is inverted in every generation. Patrik Inderbitzin[1], Periasamy Chitrampalam[2], Karunakaran Maruthachalam[1], Bo-Ming Wu[3], Krishna Subbarao[1]. 1) Department of Plant Pathology, University of California-Davis, Davis, CA, USA; 2) Department of Plant Sciences, University of Arizona, Tucson, AZ, USA; 3) Department of Plant Pathology, China Agricultural University, 2 West Yuanmingyuan Rd., Haidian District, Beijing, China.

Sclerotinia sclerotiorum is a filamentous ascomycete in the *Sclerotiniaceae* (Pezizomycotina) and a necrotrophic pathogen of more than 400 hosts worldwide, including many important agricultural crops. In California, the biggest lettuce producer in the United States, *S. sclerotiorum* is a causal agent of lettuce drop that reduces overall annual lettuce yield by 15%. Little is known about the details of sexual reproduction in *S. sclerotiorum*, but the structure of the *S. sclerotiorum* mating type locus *MAT*, the master regulator of sexual reproduction in ascomycetes, has previously been reported. As in other homothallic (self-fertile) ascomycetes, *S. sclerotiorum MAT* contains both idiomorphs (divergent alleles) fused end-to-end at a single locus. Using 283 isolates from lettuce in California and from other states and hosts, we investigated the diversity of *S. sclerotiorum MAT*, and identified a novel version of *MAT* that differed by a 3.6-kb inversion and was designated Inv+, as opposed to the previously known *S. sclerotiorum MAT* that lacked the inversion and was Inv-. The inversion affected three of the four *MAT* genes: *MAT1-2-1* and *MAT1-2-4* were inverted and *MAT1-1-1* was truncated at the 3'-end. Expression of *MAT* genes differed between Inv+ and Inv- isolates. In Inv+ isolates, only one of the three *MAT1-2-1* transcript variants of Inv- isolates was detected, and the alpha1 domain of Inv+ *MAT1-1-1* transcripts was truncated. Both Inv- and Inv+ isolates were self-fertile, and the inversion segregated in a 1:1 ratio regardless of whether the parent was Inv- or Inv+. This suggested the involvement of a highly regulated process in maintaining equal proportions of Inv- and Inv+, likely associated with the sexual state. The MAT inversion region, defined as the 3.6-kb *MAT* inversion in Inv+ isolates and the homologous region of Inv- isolates, was flanked by a 250-bp inverted repeat on either side. The 250-bp inverted repeat was a partial *MAT1-1-1* that through mediation of loop formation and crossing over, may be involved in the inversion process. Inv+ isolates were widespread, and in California and Nebraska constituted half of the isolates examined. We speculate that a similar inversion region may be involved in mating type switching in the filamentous ascomycetes *Chromocrea spinulosa*, *Sclerotinia trifoliorum* and in certain *Ceratocystis* species.

Friday, March 15 3:00 PM–6:00 PM
Fred Farr Forum

Oxidative Stress, ROS Signaling and Adaptation to Hypoxia

Co-chairs: Geraldine Butler and Barry Scott

Transcriptional regulatory networks controlling the early hypoxic response in Candida albicans. A. Nantel, M. van het Hoog, A. Sellam, C. Beaurepaire, F. Tebbji, M. Whiteway. National Research Council of Canada, Montreal, Quebec, Canada.

The ability of Candida albicans to colonize or invade multiple host environments requires that it rapidly adapts to different conditions. Our group has been exploiting ChIP-chip and transcription profiling technologies, together with computer modeling, to provide a better understanding of select transcription factor (TF) networks. We used DNA microarrays to measure the changes in transcriptional profiles that occur immediately following the transfer of C. albicans to hypoxic growth conditions. The impressive speed of this response is not compatible with current models of fungal adaptation to hypoxia that depend on the inhibition of sterol and heme biosynthesis. Functional interpretation of these profiles was achieved using Gene Set Enrichment Analysis, a method that determines whether defined groups of genes exhibit a statistically significant bias in their distribution within a ranked gene list. The Sit4p phosphatase, Ccr4p mRNA deacetylase and Sko1p TF were identified as novel regulators of the early hypoxic response. While cells mutated in these regulators exhibit a delay in their transcriptional responses to hypoxia their ability to grow in the absence of oxygen is not impeded. Promoter occupancy data on 26 TFs was combined with the profiles of 375 significantly-modulated target genes in a Network Component Analysis (NCA) to produce a model of the dynamic and highly interconnected TF network that controls this process. The NCA also allowed us to observe correlations between temporal changes in TF activities and the expression of their respective genes, thus allowing us to identify which TFs are potentially subjected to post-transcriptional modifications. The TF network is centered on Tye7p and Upc2p which are associated with many of the genes that exhibit the fastest and strongest up regulations. While Upc2p only associates with downstream promoters, Tye7p is acting as a hub, its own promoter being bound by itself and 7 additional TFs. Rap1 and Ahr1 appear to function as master regulators since they bind to a greater proportion of TF gene promoters, including those of Upc2p and Tye7p. Finally, Cbf1p, Mrr1p and Rap1p show the greatest numbers of unique gene targets. The high connectivity of these models illustrates the challenges that lie in determining the individual contributions of specific TFs.

Proteomic analysis of the hypoxic response of the human-pathogenic fungus *Aspergillus fumigatus*. Olaf Kniemeyer[1,4,5], Kristin Kroll[1,5], Vera Pähtz[1,4,5], Martin Vödisch[1,5], Falk Hillmann[1,5], Kirstin Scherlach[2], Martin Roth[3], Christian Hertweck[2], Axel A. Brakhage[1,5]. 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 2) Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 3) Bio Pilot Plant, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 4) Integrated Research and Treatment Center, Center for Sepsis Control and Care Jena, University Hospital (CSSC), Jena, Germany; 5) Department of Microbiology and Molecular Biology, Friedrich Schiller University Jena, Beutenbergstr. 11a, 07745 Jena, Germany.

Aspergillus fumigatus is a ubiquitous, filamentous fungus which may cause a broad spectrum of disease in the human host, ranging from allergic or locally restricted infections to invasive mycoses. The most fatal *A. fumigatus* disease, invasive aspergillosis occurs in patients who are severely immunocompromised and is characterized by a high mortality. During the course of the infection *A. fumigatus* has to cope with several kinds of stress conditions including low oxygen levels (hypoxia). Just recently it was shown that hypoxia adaptation is an important virulence attribute of *A. fumigatus*. To identify novel hypoxia-sensing and adapting pathways we have characterized the changes of the *A. fumigatus* proteome in response to short (3-24 hours) and long periods (7-10 days) of hypoxia (1% O_2). To maintain reproducible culture conditions, an oxygen-controlled fermenter was used. During long-term cultivation under hypoxia, proteins involved in glycolysis, the pentose phosphate shunt, amino acid biosynthesis, NO-detoxification and respiration showed an increased level. In contrast, proteins involved in sulfate assimilation and acetate activation were down-regulated. Strikingly, hypoxia also induced biosynthesis of the secondary metabolite pseurotin A. The proteomic response of *A. fumigatus* to short periods of hypoxia showed some similarities, but also marked differences: The level of glycolytic, NO-detoxifying and amino acid biosynthesis enzymes increased under both hypoxic growth conditions. However, the abundance of enzymes of the pentose-phosphate pathway decreased, whereas enzymes involved in ethanol fermentation significantly increased. To get a deeper knowledge about the specific role of metabolic pathways in adaptation to hypoxia, we have started to characterize candidate genes for their role in hypoxia by generating deletion mutants. First data will be presented and discussed.

Fgap1-mediated response to oxidative stress in trichothecene-producing *Fusarium graminearum*. M. Montibus, N. Ponts, E. Zehraoui, F. Richard-Forget, C. Barreau. INRA, UR1264-MycSA, BP81, F-33883 Villenave d'Ornon, France.

The filamentous fungus *Fusarium graminearum* infects cereals and corn. It is one of the main causal agent of "*Fusarium* Head Blight" and "Maize Ear Rot". During infection, it produces mycotoxins belonging to the trichothecenes family that accumulate in the grains. Although the biosynthetic pathway involving specific *Tri* genes has been elucidated, the global regulation of toxin biosynthesis remains enigmatic. It is now established that oxidative stress modulates the production of toxins by *F. graminearum*. H_2O_2 added in liquid cultures of this fungus enhances trichothecenes accumulation and increases *Tri* genes expression. Our working hypothesis is that a transcription factor regulates redox homeostasis, and is involved in *Tri* genes regulation. In the yeast *Saccharomyces cerevisiae*, the transcription factor Yap1p mediates response to oxidative stress *via* nuclear re-localization and activation of genes coding for detoxification enzymes. In this study, we investigate the role of Yap1p homolog in *F. graminearum*, Fgap1, in response to oxidative stress and its eventual role in the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in *F. graminearum* were constructed. We cultured these mutants in GYEP liquid medium supplemented with H_2O_2 to evaluate their sensitivity to oxidative stress and analyse their toxin production. The nuclear localization of constitutively activated Fgap1p as well as wild-type Fgap1p under oxidative stress by H_2O_2 was analyzed. Expression profiles of genes encoding oxidative stress response enzymes potentially controlled by Fgap1p and of genes involved in the biosynthesis of type B trichothecenes were analyzed by Q-RT-PCR. Trichothecene accumulation is strongly enhanced in the deleted strain, with an increase in *Tri* genes expression. On the other hand, *Tri* genes expression and toxin accumulation are drastically repressed in the mutant in which Fgap1p is constitutively activated. Moreover, the level of expression of two genes encoding catalases is modulated in both mutants. The involvement of Fgap1 in other types of stress has also been investigated. In particular, cadmium and osmotic stress affect growth in the deleted strain.

The role of NADPH oxidases in *Neurospora crassa* cell fusion. Nallely Cano-Dominguez[1], Ernestina Casto-Longoria[1], Jesus Aguirre[2]. 1) Departamento de Microbiologia, CICESE, Ensenada, Baja California, Mexico; 2) Departamento de Biologia Celular y Desarrollo. Instituto de Fisiologia Celular UNAM, Mexico City, D.F. Mexico.

Hansberg and Aguirre proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by the action of certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production of superoxide by transferring electrons from NADPH to oxygen. *Neurospora crassa* contains the NADPH oxidases NOX-1 and NOX-2 and a common regulatory subunit NOR-1. NOX-2 is essential for ascospore germination, while NOX-1 is required for sexual and asexual development, polar growth and cell fusion. NOR-1 is essential for all these NOX functions. We have found that a functional NOR-1::GFP fusion is localized throughout the cytoplasm, enriched at the hyphal tip and sometimes in aggregates. This suggests that the functional NOX complexes are probably not localized at the plasma membrane. Up to now NOX function in fungi has been evaluated in mutants that completely lack NOX proteins. We generated nox-1 alleles that result in NOX-1 proteins carrying substitutions of proline 382 by histidine or cysteine 542 by arginine, which affect NADPH-binding. Equivalent mutations in phagocytic Nox2/gp91phox do not affect protein stability but completely lack oxidase activity. P382H and C542R mutants did not produce sexual fruiting bodies and showed a decreased growth and differentiation of aerial mycelia, without affecting production of conida. These results indicate that sexual development depends on ROS production by NOX-1, whereas during asexual differentiation NOX-1 plays an important role independently of its catalytic activity. Dnox-1, Dnor-1, P382H NOX-1 and C542R NOX-1 mutants were all able to produce some conidial anastomosis tubes (CATs) but they were unable to complete cell-cell fusion. All these mutants are also impaired in vegetative hyphae-hyphae fusion, which might explain the growth defects in Dnox-1 and Dnor-1 strains. CATs production is delayed in the presence of antioxidant N- acetyl cystein (NAC) and Dsod-1 strains show an increase in CATs fusions. The results suggest that some ROS may be implicated in signaling CATs homing and vegetative fusion.

Peroxiredoxins in ROS responses -Why evolve peroxidases that are inactivated by peroxides? Alison M. Day, Jonathon D Brown, Sarah R Taylor, Jonathan D Rand, Brian A Morgan, Elizabeth A Veal. Inst Cell & Molecular Biosciences, Newcastle Univ, Newcastle Tyne, United Kingdom.

Peroxiredoxins (Prx) are extremely abundant antioxidant enzymes with important roles in protecting against oxidative stress, ageing and cancer.The thioredoxin peroxidase activity of eukaryotic typical 2-Cys Prx detoxifies hydrogen peroxide but, enigmatically, is highly sensitive to inactivation by peroxide-induced hyperoxidation of a catalytic cysteine residue. It has been proposed that hyperoxidation might allow hydrogen peroxide to act as a signal and/or promote an alternative activity of Prx as a chaperone [1, 2]. However, any advantage to be gained by inhibiting the thioredoxin peroxidase activity and preventing Prx from removing peroxides under oxidative stress conditions has remained obscure. The fission yeast Schizosaccharomyces pombe contains a single 2-Cys Prx, Tpx1. Our previous work has established that, counterintuitively, Tpx1 is vital for adaptive transcriptional responses to hydrogen peroxide due to essential roles in the hydrogen peroxide-induced activation of the p38/JNK/Hog1-related MAPK Sty1 and AP-1-like transcription factor Pap1 [3, 4]. In seeking to understand why the thioredoxin peroxidase activity of Tpx1 should be important for Pap1 activation, we have identified that Tpx1 is the major cellular substrate for thioredoxin. Accordingly, in hydrogen peroxide-treated cells, Tpx1 competitively inhibits the activity of thioredoxin towards other substrates, including Pap1, and the methionine sulphoxide reductase A, Mxr1. Consequently, we show that the oxidative inactivation of the thioredoxin peroxidase activity of Tpx1 is important to maintain active Mxr1, repair oxidative protein damage and maintain cell viability following exposure to toxic levels of hydrogen peroxide [5]. Based on these discoveries in yeast, we propose that an important function for the reversible hyperoxidation of eukaryotic 2-Cys Prx is to regulate thioredoxin and thus thioredoxin-mediated signalling and repair processes. I will present further data supporting this conclusion and discuss its implications for hydrogen peroxide signal transduction.

NADPH oxidases regulate septin-mediated cytoskeletal re-modeling during plant infection by the rice blast fungus Magnaporthe oryzae. Lauren S. Ryder[1], Yasin F. Dagdas[1], Thomas A. Mentlak[1], Michael J Kershaw[1], Martin Schuster[1], Christopher R Thornton[1], Jisheng Chen[2], Zonghua Wang[2], Nicholas J Talbot[1]. 1) Dept Biosciences, Univ Exeter, Exeter, United Kingdom; 2) Fujian agricultural university.

NADPH oxidases (Nox) are flavoenzymes that function by transferring electrons across biological membranes to catalyze reduction of molecular oxygen to superoxide. In animal cells, Nox enzymes are implicated in cell proliferation, cell signalling and apoptosis, while in plants Nox are necessary for programmed cell death, the response to environmental stresses, pathogen infection, and polarised growth of root hairs. In filamentous fungi, Nox are necessary for cellular differentiation during sexual reproduction and for developmental processes that involve transitions from non-polarised to polarised cell growth, such as tissue invasion by mutualistic and pathogenic fungi, and fungal virulence. The underlying function of Nox enzymes in these diverse developmental processes remains unclear. The rice blast fungus Magnaporthe oryzae infects plants with a specialized cell called an appressorium, which uses turgor to drive a rigid penetration peg through the rice leaf cuticle. Here, we show that NADPH oxidases (Nox) are necessary for septin-mediated re-orientation of the dynamic F-actin cytoskeleton to facilitate cuticle rupture and plant cell invasion. We report that the Nox2-NoxR complex spatially organises a heteroligomeric septin ring at the appressorium pore, required for assembly of a toroidal F-actin network at the point of penetration peg emergence. Maintenance of the cortical F-actin network during plant infection independently requires Nox1, a second NADPH oxidase, which is necessary for penetration hypha elongation. Organisation of F-actin and septins in appressoria are disrupted by application of anti-oxidants, while latrunculin-mediated depolymerisation of appressorial F-actin is competitively inhibited by reactive oxygen species (ROS), providing evidence that regulated synthesis of ROS by fungal NADPH oxidases directly controls septin and F-actin dynamics.

Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont *Epichloë festucae*. Gemma M. Cartwright, Barry Scott, Yvonne Becker. Molec Biosci, Massey Univ, Palmerston Nth, New Zealand.

Reactive oxygen species (ROS) are emerging as important regulators required for the successful establishment and maintenance of the mutualistic association between the fungal endophyte *Epichloë festucae* and its grass host *Lolium perenne*. The generation of reactive oxygen species (ROS) by the fungal NADPH oxidase, NoxA has previously been shown to regulate hyphal growth of *E. festucae* in planta; a result that has led to the hypothesis that fungal-produced ROS are key second messengers in the symbiosis. However, the highly reactive nature of these molecules dictates that cells possess efficient sensing mechanisms to maintain ROS homeostasis and prevent oxidative damage to cellular components. The *Saccharomyces cerevisiae* Gpx3-Yap1 and *Schizosaccharomyces pombe* Tpx1-Pap1, two-component H_2O_2 sensors, serve as model redox relays for coordinating the cellular response to ROS. While proteins related to the Yap1 and Pap1 basic-leucine zipper (bZIP) transcription factors have been identified in a number of filamentous fungi, the components involved in the upstream regulation remain unclear. This study investigated the role of the *E. festucae* Yap1 homologue, YapA, and putative upstream activators GpxC and TpxA, homologues of Gpx3 and Tpx1, respectively, in responding to ROS. YapA is involved in responding to ROS generated at the wound site following inoculation into ryegrass seedlings. However, deletion of *yapA* did not impair host colonization indicating redundancy in systems used by *E. festucae* to sense and respond to plant-produced ROS. In culture, deletion of *E. festucae yapA*, renders the mutants sensitive to only a subset of ROS and this sensitivity is influenced by the stage of fungal development. In contrast to the H_2O_2-sensitive phenotype widely reported for fungi lacking the Yap1-like protein, the *E. festucae yapA* mutant maintains wild-type mycelial resistance to H_2O_2 but conidia of the yapA mutant are very sensitive to H_2O_2. Using a degron-tagged GFP-CL1 as a reporter, we found YapA is required for the expression of the spore specific catalase, *catA*. Moreover, YapA is activated by H_2O_2 independently of both GpxC and TpxA, suggesting a novel mechanism of regulation exists in *E. festucae*. This work provides a comprehensive analysis of the role and regulation of the AP-1 transcription factor pathway in a filamentous fungal species.

Interaction between phenolic and oxidant signaling in *Cochliobolus heterostrophus*. Benjamin A Horwitz[1], Samer Shalaby[1], Olga Larkov[1], Mordechai Ronen[2], Sophie Lev[3]. 1) Department of Biology, Technion - IIT, Haifa, Israel; 2) Department of Plant Science, Tel Aviv University, Ramat Aviv, Israel; 3) Centre for Infectious Diseases and Microbiology, University of Sydney at Westmead Hospital, Westmead, NSW 2145, Australia.

The transcription factor ChAP1 is an ortholog of yeast YAP1 in the maize pathogen *Cochliobolus heterostrophus*. ChAP1 migrates to the nucleus upon exposure to oxidative stress, inducing antioxidant genes such as thioredoxin and glutathione reductase [1]. ChAP1 also localizes to nuclei on contact with the leaf and during invasive growth. Though reactive oxygen species are encountered on the host, ChAP1 nuclear retention can occur without oxidative stress. One of the signals responsible is provided by phenolic compounds [1-3]. Using a genetically-encoded ratiometric reporter of the redox state, we showed that leaf extract and phenolics, despite their antioxidant properties, promote nuclear accumulation of ChAP1. To study this dual role of ChAP1 we identified genes expressed in response to phenolics. Intradiol dioxygenase *CCHD1* is rapidly upregulated, independent of ChAP1 [2]. Coumaric acid caused rapid and simultaneous upregulation of most of the b-ketoadipate pathway genes. Deletion of *CCHD1* provided genetic evidence that protocatechuic acid is an intermediate in catabolism of many aromatics [3]. The activity of a structure series showed complementary requirements for upregulation of *CCHD1* and ChAP1 nuclear retention. The ability to metabolize a compound and ChAP1 nuclear retention are inversely correlated. To find additional genes induced by phenolics, microarrays designed from the predicted coding sequences of the *C. heterostrophus* genome [4] were hybridized to probes made from RNA of cultures exposed to coumaric acid, or controls. Expression of about 90 genes from different pathways primarily for metabolism, for example, the b-ketoadipate, quinic acid and shikimic acid pathways, as well as transporters from different families was altered in response to coumaric acid. The ability to respond to phenolics and detoxify or metabolize them via the b-ketoadipate pathway confers an advantage to plant pathogens, and explains the presence of at least two response pathways detecting these compounds. [1] Lev et al. (2005) Eukaryot. Cell 4:443-454; [2] Shanmugam et al. (2010) Cell. Microbiol. 12:1421-1434; [3] Shalaby et al. (2012) MPMI 25: 931-940; [4] Ohm et al. (2012) PLoS Pathog 8: e1003037. Supported in part by the Israel Science Foundation. We thank Michal Levin and Itai Yanai for help with microarray hybridization.

Friday, March 15 3:00 PM–6:00 PM
Kiln

Phylogenomics

Co-chairs: Jason Stajich and Joey Spatafora

Characterizing Gene Tree Incongruence on a Genome Scale. Dannie Durand. Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Gene families evolve through gene duplication and loss, and lateral gene transfer. Reconstructing these events is a powerful approach to understanding the co-evolution of genes and species and the emergence of novel protein function. Gene duplication, loss, and transfer can all result in a gene tree that disagrees with the species tree. This incongruence can be exploited to infer the history of these events, as well as the ancestral lineage in which each event took place. This is achieved by fitting the gene family tree to the associated species tree, a process called reconciliation. I will discuss the benefits and challenges of gene tree reconciliation, with special attention to genome scale analyses. The use of gene tree reconciliation will be compared with non-phylogenetic analyses of gene family expansion and contraction. The problem of determining whether the observed incongruence is due to gene duplication, lateral transfer, or incomplete lineage sorting will also be discussed. I will present analyses of several large gene tree data sets from well-studied species lineages, as a practical demonstration of this approach. Our algorithms have been implemented in [http://www.cs.cmu.edu/~durand/Notung], a freely available software tool.

Early fungi and their carbohydrate active enzymes. Mary L. Berbee[1*], Satoshi Sekimoto[2], Joseph Spatafora[3], Timothy James[4], Teresita M. Porter[5], Rytas Vilgalys[6]. 1) Dept Botany, Univ British Columbia, Vancouver, B.C., Canada; 2) Department Of Biological Sciences, The University Of Alabama, Tuscaloosa, AL; 3) Oregon State University, Dept of Botany & Plant Pathology, 2082 Cordley Hall, Corvallis, OR; 4) University of Michigan, Dept of Ecology & Evol Biology, 830 N University, Ann Arbor, MI; 5) 16 Yachters Lane, Etobicoke, ON, Canada; 6) Biology Department 130 Science Drive, Biological Sciences Rm 137, Duke University Box 90338, Durham, NC.

Early fungi are intermingled with some of the oldest fossils from vascular plants, dated at 400 Ma. However, what the fungi were doing for their nutrition before land plants were available has been difficult to reconstruct because in phylogenies of the earliest diverging fungal lineages, saprotrophs and parasites of plants as well as animals are intermingled, and which fungal life style came first is ambiguous. We are using phylogenetic analysis of enzymes involved in carbohydrate metabolism to reconstruct the enzymatic capabilities of some of the early terrestrial fungi. Our community sequencing proposal to the US Joint Genome Institute resulted in four new genome sequences for evolutionarily divergent lineages including aquatic fungi, the chytrids and Blastocladiomycota, and zygomycetes. Analysis of the genomes suggests that cellulases and pectinases to degrade plant wall carbohydrates were already present in the earliest fungal lineages but largely lost from the zygomycetes. This implies that fungi evolved in association with the green algal/green plant lineage. Even with complete genome sequences, the branching order among the aquatic fungi and zygomycetes remains problematical, and branching order conflicts from one analysis to another. The conflicts may reflect difficulties involved in modeling evolutionary processes across lineages. Alternatively, the conflicts may indicate that fungi, like animals, underwent a 'Cambrian Explosion' perhaps facilitated by rapid expansion of nutritional resources offered by radiation of multicellular plants and animals.

Better evolution through gene clustering. Jason Slot[1], Matthew Campbell[2], Han Zhang[1], Martijn Staats[3], Jan van Kan[4], Antonis Rokas[1]. 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Botany, University of Hawai`i, Manoa, HI; 3) Biosystematics group, Wageningen University, Wageningen, The Netherlands; 4) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.

The recent availability of a large number of fungal genomes has facilitated systematic investigations of metabolic pathway evolution across the kingdom. Through combining phylogenetic and genomic techniques, we have recently examined the evolution of metabolic pathways across a well-sampled fungal phylogeny, and gained new insight into the role of metabolic gene clusters in fungal evolution. The occasional occurrence of horizontal gene transfer of entire pathways between distantly related fungi via gene clusters suggests that fungal species have access to larger pan-genomes than previously thought. Furthermore, analysis of gene cluster decay suggests these transfers are underestimated by analyses of single strains, and that evolution within clustered pathways is constrained by natural selection. Increased evolvability in fungi is also implied by the discovery of chromosomal loci that maintain large alternative secondary metabolite gene clusters within recombining lineages. Together, these phylogenomic analyses in fungi illustrate a multi-faceted role of gene clustering in fungal evolution.

Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales. C. Alisha Owensby, Kathryn E. Bushley, Joseph W. Spatafora. Botany & Plant Pathology, Oregon State University, Corvallis, OR.

Hypocreales is an order characterized by a dynamic evolutionary history of interkingdom host jumping, with members that parasitize animals, plants, and other fungi. The monophyly of taxa attacking members of the same kingdom is not supported by molecular phylogenetics, however. For example, *Trichoderma spp.* and *Elaphocordyceps spp.* are both mycoparasitic, but are members of different families within Hypocreales, Hypocreaceae and Ophiocordycipitaceae, respectively. In fact, both genera are more closely related to insect pathogens, than they are to each other. Multiple species of *Trichoderma* have sequenced genomes, and recently genomes of several insect pathogens in Hypocreales have been completed (e.g. *Metarhizium spp.* and *Tolypocladium inflatum*). The genus *Elaphocordyceps* represents a unique clade within Hypocreales, because whereas most species in the family Ophiocordycipitaceae are insect pathogens, most *Elaphocordyceps* parasitize truffles of the ectomycorrhizal genus *Elaphomyces* [Eurotiales, Ascomycota]. To compare genes of a truffle pathogen with hypocrealean insect pathogens and mycoparasites, we sequenced the genome of *Elaphocordyceps ophioglossoides*. Our draft assembly of the *E. ophioglossoides* genome is ~32 MB and has 10,779 gene models, 36 of which are predicted to produce secondary metabolites. We have identified three very large genes in *E. ophioglossoides* related to peptaibol producing nonribosomal peptide synthetase (NRPS) genes. Peptaibols, which disrupt osmoregulation by forming ion channels through lipid bilayers, have antibiotic and antifungal activity and are best described in *Trichoderma spp*. *E. ophioglossoides* and its beetle-pathogenic congener, *T. inflatum*, both possess three putative peptaibol synthetases which we identified through analysis of NRPS adenylation domains. Of the three peptaibol-specific domain clades, one is predicted to encode for the nonproteinogenic a-aminoisobutryic acid residues. We also show that, despite being very closely related, *E. ophioglossoides* and *T. inflatum* each possess

three different peptaibol-like genes, only two of which appear to be located in syntenic regions. The current distribution of fungi possessing peptaibol genes is restricted to mycoparasitic lineages of Hypocreales and is generating hypotheses about the role of secondary metabolites in mycoparasitism.

Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum. Robert Riley[1], Asaf Salamov[1], Robert Otillar[1], Kirsten Fagnan[1], Bastien Boussau[3], Daren Brown[4], Bernard Henrissat[5], Anthony Levasseur[5], Benjamin Held[6], Laszlo Nagy[2], Dimitris Floudas[2], Emmanuelle Morin[7], Gerard Manning[8], Scott Baker[9], Robert Blanchette[6], Francis Martin[7], David Hibbett[2], Igor Grigoriev[1]. 1) Joint Genome Istitute, Lawrence Berkeley National Lab, Walnut Creek, CA; 2) Clark University, Worcester, MA; 3) UC Berkeley, Berkeley, CA; 4) USDA, Peoria, IL; 5) AFMB, Marseille, France; 6) UMN, St. Paul, MN; 7) INRA, France; 8) Salk Institute, La Jolla, CA; 9) Pacific Northwest National Lab, Richland, WA.

Fungi of the phylum Basidiomycota (basidiomycetes), make up some 37% of the described fungi, and are important in forestry, agriculture, medicine, and bioenergy. This diverse phylum includes symbionts, pathogens, and saprobes including wood decaying fungi. To better understand the diversity of this phylum we compared the genomes of 35 basidiomycete fungi including 6 newly sequenced genomes. The genomes of basidiomycetes span extremes of genome size, gene number, and repeat content. A phylogenetic tree of Basidiomycota was generated using the Phyldog software, which uses all available protein sequence data to simultaneously infer gene and species trees. Analysis of core genes reveals that some 48% of basidiomycete proteins are unique to the phylum with nearly half of those (22%) comprising proteins found in only one organism. Phylogenetic patterns of plant biomass-degrading genes suggest a continuum rather than a sharp dichotomy between the white rot and brown rot modes of wood decay among the members of Agaricomycotina subphylum. There is a correlation of the profile of certain gene families to nutritional mode in Agaricomycotina. Based on phylogenetically-informed PCA analysis of such profiles, we predict that that Botryobasidium botryosum and Jaapia argillacea have properties similar to white rot species, although neither has liginolytic class II fungal peroxidases. Furthermore, we find that both fungi exhibit wood decay with white rot-like characteristics in growth assays. Analysis of the rate of discovery of proteins with no or few homologs suggests the high value of continued sequencing of basidiomycete fungi.

Genome evolution of fungal pathogens from the Magnaporthe oryzae/grisea clade. Helene Chiapello[1,2], Ludovic Mallet[1,3], Cyprien Guérin[1], Gabriela Aguileta[4], François Rodolphe[1], Annie Gendrault[1], Jonathan Kreplak[3], Joelle Amselem[3], Enrique Ortega-Abboud[5], Marc-Henri Lebrun[6], Didier Tharreau[5], Elisabeth Fournier[7]. 1) INRA, UR MIG, 78352 Jouy-en-Josas, France; 2) INRA, UR BIA, 31326 Castanet-Tolosan, France; 3) INRA, URGI, 78026 Versailles, France; 4) CRG, Barcelone, Spain; 5) CIRAD, UMR BGPI, TA 54K, 34398 Montpellier, France; 6) INRA, UMR BIOGER, 78850 Thiverval-Grignon, France; 7) INRA, UMR BGPI, TA 54K, 34398 Montpellier, France.

The GEMO project aims at characterizing genomic determinants of pathogenicity and evolutionary events involved in adaptation of 9 isolates from the M. oryzae/grisea clade differing in their host specificity. Eight strains from M. oryzae species complex and one of the related species M. grisea have been sequenced and assembled. De novo structural gene annotation was carried out using Eugene (Schiex, 2001) to predict genes and REPET (Flutre, 2011) to annotate Transposable Elements (TEs) in these 9 genomes. Four of them exhibited large supplementary genomic regions potentially issued from an unknown bacterial strain of the Burkholderia genus. An original strategy based on Gotham software (Ménigaud, 2012) was used to accurately quantify these regions in all the affected genome scaffolds. Functional gene annotations were performed using InterProScan. Databases and interfaces relying on the GMOD tools (gmod.org) were set up to browse annotations and facilitate further evolutionary analyses. In order to identify gene families, the entire set of the predicted and known proteins of the M. oryzae/grisea genomes were clustered using OrthoMCL (Li, 2003). A total of 20443 clusters (15326 assigned to M.oryzae/grisea and 5117 to Burkholderia) were obtained, including 8154 clusters comprising single copy genes shared by all genomes (core genome) and variable number of genome specific gene families (305-1550). Genes encoding putative Secreted Proteins (SPs) were identified in 2522 OrthoMCL clusters (2271 of M. oryzae/grisea and 251 of Burkholderia). Further analyses regarding genome-specific and rice-infecting specific genes and SPs will be presented. OrthoMCL families were processed to infer the phylogenetic reference genealogy of the M. oryzae/grisae complex. We also evaluated the ability of individual genes to recover the same topology as that supported by most of the genes by using a recent method based on multiple co-inertia analysis (de Vienne, 2012). Most of the genes exhibit a concordant topology with the reference tree except a small set of 'outliers'. Further investigations are currently being performed to determine possible causes for incongruities. Finally, we present preliminary results regarding the comparison of TE distribution in M. oryzae/grisea species taking into account the reference genealogy of the strains.

***Leptosphaeria maculans* 'brassicae': "Transposable Elements changed my life, I feel different now".** Jonathan Grandaubert[1], Conrad Schoch[2], Hossein Borhan[3], Barbara Howlett[4], Thierry Rouxel[1]. 1) INRA-BIOGER, Thiverval-Grignon, France; 2) NCBI, National Institutes of Health, Bethesda, MD, USA; 3) AAF Saskatoon, Canada; 4) School of Botany, University of Melbourne, Australia.

The Dothideomycetes phytopathogens *Leptosphaeria maculans* and *Leptosphaeria biglobosa* form a complex of 8 species and putative subspecies suggested to have diverged "recently". In 2007, the sequencing of an isolate of *Leptosphaeria maculans* 'brassicae' (Lmb) provided the first reference genome for this fungus. The 45-Mb genome has an unusual bipartite structure, alternating large GC-equilibrated and AT-rich regions. These AT-rich regions comprise one third of the genome and are mainly composed of mosaics of truncated Transposable Elements (TEs) postulated to have "invaded" the genome 5-10 MYA; they also comprise 5% of the predicted genes of which 20% encode putative effectors. In these regions, both genes and TEs are affected by Repeat Induced Point mutation (RIP). To investigate when and how genome expansion took place in the evolutionary series, and the consequences it had on fungal adaptability and pathogenicity, the genomes of five members of the species complex showing contrasted host range and infection abilities were sequenced. In silico comparison of the reference genome with that of 30-32-Mb genome of *L. maculans* 'lepidii' (Lml), *L. biglobosa* 'brassicae', *L. biglobosa* 'thlaspii' and *L. biglobosa* 'canadensis', showed these species have a much more compact genome with a very low amount of TEs (<4%). The TE annotation allowed us to identify 121 TE families, all RIP-affected including the expected presence of lineage-specific TEs. Unexpectedly, two of the most expanded TE families in Lmb have been present in the Dothideomycete lineage for 100 million years. This questions how these families, while they have been anciently RIPped, managed to expand recently in Lmb. Interestingly, the comparison between the TE-rich genome of Lmb and the TE-poor genome of Lml, estimated to have diverged 5.5 MYA, indicated a nearly perfect synteny at the chomosomal level, suggesting low incidence of TE expansion on genome reorganisation. The gene annotation produced a similar gene number in each genome (~11000), but compared to the reference genome, less than 20% of the effector genes and 50% of other genes in AT-rich regions are present in the other genomes, suggesting that TEs were key players in gene innovation and that the genome environment promoted rapid sequence diversification and selection of genes involved in pathogenicity.

Comparing comparative "omics" in *Coccidioides spp*. Emily A. Whiston, John W. Taylor. Plant & Microbial Biology, U.C. Berkeley, Berkeley, CA.

The mammalian pathogens *Coccidioides immitis* and *C. posadasii* are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, all of *Coccidioides*' closest known relatives are non-pathogenic. In this project, we are interested in genome changes between the *Coccidioides* lineage and its relatives, and how these changes compare to recently published comparative and population genomics, and transcriptomics studies in *Coccidioides*. *Coccidioides* and its closest sequenced relative, *Uncinocarpus reesii*, are estimated to have diverged 75-80 million years ago. Here, we have sequenced the genomes of four species more closely related to *Coccidioides* than *U. reesii*: *Byssoonygena ceratinophila, Chrysosporium queenslandicum, Amauroascus niger* and *A. mutatus*. For each of these four species, we prepared genomic DNA Illumina sequencing libraries; the resulting genome assemblies ranged from 23-34Mb, with N50 of 90kb-205kb. Predicted genes were confirmed by RNAseq; the total number of genes ranged from 8,179-9,184. We assessed individual gene gain/loss, and gene family expansion/contraction in *Coccioides* using these new genomes and other recently published genomes from the Onygenales order, including the yeast-forming dimorphic pathogens *Histoplasma* and *Paracoccidioides*, and the dermatophytes *Microsporum* and *Trichopyton*. We have compared these results to genes identified in recently published *Coccidioides* "omics" studies that show evidence of positive selection, introgression and/or differential expression.

Friday, March 15 3:00 PM–6:00 PM
Nautilus

Synthetic Biology

Co-chairs: Nancy Keller and Peter Punt

Engineering Aspergillus oryzae for high level production of L-malic acid. Debbie S Yaver[1], S. Brown[2], A. Berry[2]. 1) Expression Technology, Novozymes, Inc., Davis, CA; 2) Microbial Physiology, Novozymes, Inc., Davis, CA.

In the last decade, there has been widespread interest and investment in developing processes for the production of bulk and specialty chemicals from renewable feedstocks by fermentation. During this period, Novozymes has successfully developed technology for production of a specialty molecule (hyaluronic acid) by Bacillus fermentation and has been very active in developing technologies for the production of bulk chemicals by metabolic engineering and fermentation using several different microorganisms. An example of the latter is L-malic acid. In the literature it is reported that some wild-type Aspergillus strains produce high levels of malic acid under specific cultivation conditions. Concentrations up to 113 g/L malate (94% w/w from glucose) reported for A. flavus in fed-batch fermentations (Battat, et. al., 1991. Biotechnol. Bioeng. 37:1108-1116). The goal of our work was to improve malic acid production in the natural malic acid producing filamentous fungus Aspergillus oryzae NRRL 3488 by overexpression of cloned genes and classical mutagenesis. More than 75 different recombinant strains were tested containing combinations of overexpression of genes as well as deletions. A high through put screen was developed and used to screen mutagenized strains. Combined genetic engineering and mutagenesis/HTS was used to increase the malic acid production rate of A. oryzae NRRL3488 by 4-fold with final C4 acid totals of 340 g/l at 8 days in lab scale fermentations.

When synthetic biology meets metabolic engineering: *in vivo* pathway assembly in *Saccharomyces cerevisiae*. Niels Kuijpers[1,2], Daniel Solis Escalante[1,2], Jack T. Pronk[1,2,3], Jean-Marc Daran[1,2,3], Pascale Daran-Lapujade[1,2]. 1) Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands; 2) Kluyver Centre for Genomics of Industrial Fermentation, PO Box 5057, 2600 GA Delft, The Netherlands; 3) Platform Green Synthetic Biology, Julianalaan 67, 2628 BC Delft, The Netherlands.

The yeast *Saccharomyces cerevisiae* is a powerful and versatile workhorse intensively exploited for a wide range of biotechnological applications. Besides the large scale production of endogenous products, such as the biofuel ethanol, *S. cerevisiae* has been genetically engineered to produce many heterologous compounds, including half of the worldwide insulin market. The past decade has been marked by the conversion of *S. cerevisiae* into a complex cell factory with remarkable new capabilities such as the production of the anti-malaria drug precursor artemisinic acid. The ever-increasing demand for cheap and sustainable production of complex molecules combined with its attractiveness as a host for pathway engineering will inevitably intensify the exploitation of *S. cerevisiae* as cell factory in the future. Even in the genetically accessible bakers yeast, expression of dozen of genes is still largely based on laborious classical techniques involving successive restrictions and ligations, complemented with the creative application of PCR. However, the increasing size and complexity of today's constructs in metabolic engineering has made design and construction of plasmids by these classical techniques increasingly complicated and time consuming. Although uncovered nearly three decades ago, the high efficiency of *S. cerevisiae* homologous recombination is only beginning to reveal its full potential for the assembly of large DNA constructs (Gibson et al., 2008). *In vivo* assembly in yeast is predicted to have a large impact on laboratory practices, ranging from simple plasmid construction to engineering of complex pathways via automated high-throughput strain construction. Despite those promising prospects, *in vivo* assembly has not yet become a standard technique in most academic laboratories. This offers unique possibilities for standardization and, simultaneously, for further optimization. In the present work we describe an approach designed to improve the efficiency of *in vivo* assembly and to make a robust, versatile *in vivo* assembly strategy for multi-component plasmids. As a proof of principle, the method was used to assemble a 21 kb plasmid from 9 overlapping fragments, using only PCR and yeast transformation. Gibson D.G. et al. (2008), Science, 319, 1215-1220.

Analysis of the intracellular galactoglycom of Trichoderma reesei grown on lactose. Levente Karaffa[1], Leon Coulier[2], Erzsébet Fekete[1], Karin M. Overkamp[2], Irina S. Druzhinina[3], Marianna Mikus[3], Bernhard Seiboth[3], Levente Novák[4], Peter J. Punt[2], Christian P. Kubicek[3]. 1) Department of Biochemical Engineering, University of Debrecen, H-4032, Debrecen, Hungary; 2) TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands; 3) Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Gumpendorferstrasse 1a, A-1060 Wien, Austria; 4) Department of Colloid and Environmental Chemistry, Faculty of Science and Technology, University of Debrecen, H-4032, Debrecen, Hungary.

Lactose (1,4-0-b-D-galactopyranosyl-D-glucose) is used as a soluble carbon source for the production of cellulases and hemicellulases for - among other purposes - in the biofuel and biorefinery industries. However, the mechanism how lactose induces cellulase formation in T. reesei is still enigmatic. Previous results raised the hypothesis that intermediates from the two D-galactose catabolic pathway may give rise to the accumulation of intracellular oligogalactosides that could act as inducer. We have therefore used HPAEC-MS to study the intracellular galactoglycome of T. reesei during growth on lactose, in T. reesei mutants impaired in galactose catabolism, and in strains with different cellulase productivity. Lactose, allo-lactose and lactulose were detected in the highest amounts in all strains, and two trisaccharides (Gal-b-1,6-Gal-b-1,4-Glc/Fru; and Gal-b-1,4-Gal-b-1,4-Glc/Fru) also accumulated to significant levels. D-Glucose and D-galactose, as well as two further oligosaccharides (Gal-b-1,3/1,4-Gal; Gal-b-1,2/1,3-Glc) were only detected in minor amounts, In addition, one unknown disaccharide and four trisaccharides were also detected. The unknown hexose disaccharide to correlate with cellulase formation in the improved mutant strains as well as the galactose pathway mutants, and Gal-b-1,4-Gal-b-1,4-Glc and two other unknown hexose trisaccharides to correlate with cellulase production only in the pathway mutants, suggesting that these compounds could be involved in cellulase induction by lactose.

Novel transcriptomics approaches for metabolic pathway engineering target identification in *Aspergillus*. Peter J. Punt, Martien Caspers, Marvin Steijaert, Eric Schoen, Machtelt Braaksma. Microbiology, TNO, Zeist, Netherlands.

Among filamentous fungi *Aspergillus sp*. are well known production host for several organic acids. These acids, traditionally being food ingredients, more recently have gained attention as platform or building-block chemicals. These chemicals, currently mostly produced based on petrochemistry, are the starting point for the production of a wide variety of materials, such as resins, plastics, etc. Production of these compounds via biobased routes will be a major contribution towards a Biobased Economy. For the production of these bulk compounds robust host organisms are required, suitable for using low cost lignocellulose-based feedstocks, resistant against adverse conditions due to inhibitory feedstock compounds and capable of coping with high product concentrations. *A. niger* was shown to fulfill most of these prerequisites (Rumbold et al., 2009).Based on the extended molecular genetic toolkit systems biology approaches were developed for *A. niger* and other fungi (e.g. Braaksma et al., 2010). These approaches were followed towards production of these platform chemicals in *A. niger*, as demonstrated by the example of itaconic acid (Li et al., 2011, 2012). The recent development of novel high throughput sequence methods has led to new much more efficient transcriptomics approaches such as RNAseq. Combination of these approaches with novel experimental design and statistical methods for targetgene identification in metabolic pathway engineering will be illustrated. Rumbold, K., van Buijsen, H.J.J., Overkamp, K.M., van Groenestijn, J.W., Punt, P.J., Werf, M.J.V.D. (2009) Microbial production host selection for converting second-generation feedstocks into bioproducts. Microbial Cell Factories 8, art. no. 64 Braaksma, M., van den Berg, R.A., van der Werf, M.J., Punt, P.J. (2010) A Top-Down Systems Biology Approach for the Identification of Targets for Fungal Strain and Process Development. In: Cellular and Molecular Biology of Filamentous Fungi. Eds: K.A. Borkovich & D.J. Ebbole ASM Press, Washington DC. pp. 25-35 Li, A., van Luijk, N., ter Beek, M., Caspers, M., Punt, P., van der Werf, M. (2011) A clone-based transcriptomics approach for the identification of genes relevant for itaconic acid production in *Aspergillus*. Fungal Genetics and Biology 48 (6), pp. 602-611.

A new method for gene mining and enzyme discovery. Y. Huang[1,2,3], P. Busk[1], M. Grell[1], H. Zhao[2,3], L. Lange[1]. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610041, PR China; 3) University of the Chinese Academy of Sciences, Beijing 100049, PR China.

Peptide pattern recognition (PPR) is a non-alignment based sequence analysis principle and methodological approach, which can simultaneously compare multiple sequences and find characteristic features. This method has improved the understanding of structure/function relationship for enzymes within the CAZY families, which would make it easier to predict the potential function of novel enzymes, creating bigger promises for industrial purposes. *Mucor circinelloides*, member of the former subdivision Zygomycota, can utilize complex polysaccharides such as wheat bran, corncob, xylan, CMC and avicel as substrate to produce plant cell wall degrading enzymes. Although the genome of *M. circinelloides* has been sequenced, only few plant cell wall degrading enzymes are annotated in this species. In the present project, PPR was applied to analyze glycoside hydrolase families (GH family) and mining for new GH genes in *M. circinellolides* genome. We found 19 different genes encoding GH3, GH5, GH6, GH7, GH9, GH16, GH38, GH43, GH47 and GH125 in the genome. Of the three GH3 encoding genes found, one was predicted by PPR to encode a b-glucosidase. We expressed this gene in *Pichia pastoris* and found that the recombinant protein has high b-glucosidase activity (4884 U/mL). In this work, PPR provided targeted short cut to discovery of enzymes with a specific activity. Not only could PPR pinpoint genes belonging to different GH families but it did also predict the enzymatic function of the genes.

Increased production of fatty acids and triglycerides in *Aspergillus oryzae* by modifying fatty acid metabolism. Koichi Tamano[1], Kenneth Bruno[2], Tomoko Ishii[1], Sue Karagiosis[2], David Culley[2], Shuang Deng[2], James Collet[2], Myco Umemura[1], Hideaki Koike[1], Scott Baker[2], Masayuki Machida[1]. 1) National Institute of Advanced Industrial Science and Technology (AIST); 2) Pacific Northwest National Laboratory (PNNL).

Biofuels are attractive substitutes for petroleum based fuels. Biofuels are considered they do not contribute to global warming in the sense they are carbon-neutral and do not increase carbons on the globe. Hydrocarbons that are synthesized by microorganisms have potential of being used as biofuels or the source compounds. In the hydrocarbon compounds synthesized by *A. oryzae*, fatty acids and triglycerides are the source compounds of biodiesel that is fatty acid methyl ester. We have increased the production by modifying fatty acid metabolism with genetic engineering in *A. oryzae*. Firstly, enhanced-expression strategy was used for the increase. For four enzyme genes related to the synthesis of palmitic acid [C16:0-fatty acid], the individual enhanced-expression mutants were made. And the fatty acids and triglycerides in cytosol were assayed by enzyme assay kits, respectively. As a result, both fatty acids and triglycerides were most synthesized in the enhanced-expression mutant of fatty acid synthase gene at 2.1-fold and 2.2-fold more than the wild-type strain, respectively. Secondly, gene disruption strategy was used for the increase. Disruptants of several enzyme genes related to long-chain fatty acid synthesis were made individually. And one of them showed drastic increase in fatty acid synthesis. In the future, further increase in the synthesis is expected by utilizing genetic engineering in *A. oryzae*.

Molecular biological basis for statin resistance in naturally statin-producing organisms. Ana Rems, Rasmus Frandsen. DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark.

Secondary metabolites can be toxic to the organism producing them; therefore gene clusters for biosynthesis of secondary metabolites often include genes responsible for the organism's self-resistance to the toxic compounds. One such gene cluster is the compactin (ML-236B) cluster in *Penicillium solitum*. Compactin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, and is used as a precursor for production of the cholesterol-lowering drug pravastatin. The compactin gene cluster includes two genes encoding proteins that may confer the self-resistance to compactin and its secretion [1]. The *mlcD* gene encodes a putative 'HMG-CoA reductase-like protein', and *mlcE* encodes a putative efflux pump. However, the function of these two putative proteins has not yet been confirmed. We aim to elucidate the biological basis for compactin resistance in the compactin-producing organism. A codon-optimized version of the *mlcD* gene was inserted into the *Saccharomyces cerevisiae* genome. The constructed yeast strain was tested for sensitivity to lovastatin, a statin structurally similar to compactin, by growing the strain on media containing lovastatin. The strain showed an increased resistance to lovastatin compared to the wild-type strain. Furthermore, we investigated if MlcD confers the resistance by functional complementation of the endogenous HMG-CoA reductases in *S. cerevisiae*. There are two isozymes of HMG-CoA reductase in yeast, *HMG1* and *HMG2*, both involved in the sterol biosynthetic pathway, which leads to the synthesis of ergosterol. Following deletion of *HMG1* and *HMG2* genes in *S. cerevisiae*, we inserted the *mlcD* gene into the knockout mutants, and tested the resulted strains for sensitivity to lovastatin. The *HMG1* and *HMG2* knockout mutants were unable to grow on minimal media and had an increased sensitivity to lovastatin on rich media. However, insertion of the *mlcD* gene restored the growth of the yeast mutants and increased their resistance to lovastatin. These results show that MlcD complements the activity of the deleted HMG-CoA reductases, enabling synthesis of ergosterol in yeast. In addition MlcD confers statin resistance by being insensitive to the inhibiting effects of statins. Reference: [1] Abe Y., Suzuki T., Ono C., Iwamoto K., Hosobuchi M., Yoshikawa H. Mol Genet Genomics 2002, 267, 5:636-46.

Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms. Hong Luo, John S. Scott Craig, Robert M. Sgambelluri, Sung-Yong Hong, Jonathan D. Walton. Department of Energy Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824, United States.

Ninety percent of fatal mushroom poisonings are caused by alpha-amanitin and related bicyclic peptides found in some species of Amanita, Galerina, Lepiota, and Conocybe. We showed that the amatoxins (mainly amanitins) and related phallotoxins are synthesized on ribosomes in A. bisporigera and the unrelated mushroom G. marginata. The primary gene products are short (34-35 amino acid) proproteins that are initially processed by a dedicated prolyl oligopeptidase. A genome survey sequence of A. bisporigera suggested that it has a repertoire of over 40 cyclic peptides, all produced on a single biosynthetic scaffold. Members of this extended gene family are characterized by conserved upstream and downstream amino acid sequences, including two invariant proline residues, flanking a six to ten-amino acid "hypervariable" region that encodes the amino acids found in the mature toxins (or predicted toxins). The evidence indicates that A. bisporigera has evolved a combinatorial strategy that could in principle biosynthesize billions of small cyclic peptides. In order to study the other steps in amanitin biosynthesis, and to engineer novel cyclic peptides, we have developed a transformation strategy for the amanitin-producing mushroom G. marginata. This first transformation method uses Agrobacterium-mediated transformation followed by hygromycin selection. Taking advantage of this platform, we are introducing artificial toxin genes that are deliberately designed to provide insights into the pathway. The synthetic genes include those that encode the cyclic octapeptide beta-amanitin, the heptapeptides phalloidin and phallacidin, examples of the toxin gene family known from A. bisporigera but not G. marginata, and randomly generated artificial sequences. Currently, thousands of transformants have been generated through an efficient pipeline and the transformants are being analyzed for production of the expected products. If successful, the novel peptides will be screened in a number of assays including RNA polymerase (the site of action of alpha-amanitin), membrane ion channels, pathogenic bacteria, and cancer cell lines.

Friday, March 15 3:00 PM–6:00 PM
Scripps

Fungicides and Antifungal Compounds

Co-chairs: Daniele Debieu and Paul Verweij

Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*. D. A. Macdonald[1], A. E. Johns[1], M. Eberle[2], P. Bowyer[1], D. Denning[1], M. J. Bromley[1]. 1) Institute of Inflammation and Repair, Respiratory & Allergy Centre, University of Manchester, Manchester, United Kingdom; 2) Applied Microbiology, Institute for Applied Life Sciences, University of Karlsruhe, Hertzstrae 16, 76187 Karlsruhe, Germany.

Current drugs used to treat Aspergillus infections are limited and suffer from a variety of shortcomings including low efficacy, toxicity and increasing resistance. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the 'druggability' of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic to humans. Consequently most of the antimicrobials presently on the market were originally discovered by random screening of compounds against whole cell screens. A solution to this problem is to identify gene targets utilizing compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as a diploid strain lacking a single copy of a drug's target is hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80*$^{-}$*/Ku80*$^{-}$ mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated with several compounds. High-throughput methods of chemical genetic profiling by pooling multiple heterozygous KO strains into a single culture is currently being validated and preliminary data is promising. This will enable high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

Inhibition of benzoate 4-monooxygenase (CYP53A15) from *Cohliobolus lunatus* by cinnamic acid derivatives. Branka Korosec[1], Barbara Podobnik[2], Sabina Berne[3], Neja Zupanec[1], Metka Novak[1], Nada Krasevec[1], Samo Turk[4], Matej Sova[4], Ljerka Lah[1], Jure Stojan[3], Stanislav Gobec[4], Radovan Komel[1,3]. 1) National Institute of Chemistry, Ljubljana, Slovenia; 2) Lek Pharmaceuticals d.d., Verovskova 57, SI-1000 Ljubljana, Slovenia; 3) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia; 4) Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Askerceva cesta 7, SI-1000 Ljubljana, Slovenia.

Fungal infections cause huge economic losses in agriculture. Some of the major phytopathogens also cause serious, and very often lethal, infections in human and animals. Plants may be a good source of antifungals since they have to defend themselves by producing numerous secondary metabolites, such as sterols, terpens, polycosanols and phenolic compounds. Successful development of antifungal compounds, based on natural defense molecules, could prove useful in combating infectious and toxin-producing fungi in both agriculture and medicine. In recent years several promising antifungal targets have been under exploration. One of such is also fungal CYP53, member of the family of highly conserved CYP proteins, involved in detoxification of benzoate, a key intermediate in metabolism of aromatic compounds in fungi. High specificity and absence of homologue in higher eukaryotes assign CYP53A15 from the filamentous fungus *Cochliobolus lunatus* as interesting drug target. In our latest research we explored chemical properties of isoeugenol for ligand-based similarity searching, and the homology model of CYP53A15 of *Cochliobolus lunatus*, for structure-based virtual screening of a composite chemical library. Two cinnamic acid derivatives were amongst the highest scoring compounds. In the past few years, several other reports about antifungal activity of cinnamic acid derivatives have been published. In order to investigate the potential inhibitory activity on benzoate 4-monooxygenase (CYP53A15) we analyzed antifungal activity of 9 commercially available, and 10 representative cinnamic acid derivatives from our library. Furthermore, to obtain more information about structure-activity relationship 26 additional cinnamic acid esters and amides were synthesized and included in our assays. Among 45 cinnamic acid derivatives tested, 7 compounds have shown antifungal activity against *C. lunatus*, *A. niger* and *P. ostreatus* in in vivo inhibition tests. Compounds with antifungal activity were further evaluated for inhibition of CYP53A15 activity with spectral binding titration assay and HPLC. The best two inhibitors of CYP53A15 activity showed 70% inhibition at 600 mM concentration and were selected for further optimization of new lead structures.

Secretome analysis of *Trichoderma harzianum* cultivated in the presence of *Fusarium solani* cell wall or glucose. Marcelo HS Ramada[1,3], Andrei S Steindorff[1], Carlos Bloch Jr.[3], Cirano J Ulhoa[2]. 1) Brasilia University, Cell Biology Department, Brasilia, DF, Brazil; 2) Federal University of Goias, Biochemistry Department, Goiania, GO, Brazil; 3) EMBRAPA CENARGEN, Mass Spectrometry Laboratory, Brasilia, DF, Brazil.

Trichoderma harzianum is a fungus well known for its potential as a biocontrol agent of many fungal phytopathogens. The aim of this study was to evaluate the potential of *T. harzianum* ALL42 to control *Fusarium solani*, a phytopathogen fungus that causes several losses in common bean and soy crops in Brazil and to evaluate the secreted proteins of *T. harzianum* ALL42 when its spores were inoculated and incubated in culture media supplemented (TLE) or not (MM) with nitrogen sources and in the presence or not of *F. solani* cell walls (FsCW). In the absence of FsCW, the media were supplemented with glucose (GLU). *T. harzianum* was able to control the phytopathogen growth and started to sporulate in its area after 7 days in a dual culture assay, indicating that it had successfully parasitized the host. *T. harzianum* was able to grow in TLE+FsCW, MM+FsCW, TLE+GLU, but unable to grow in MM+GLU. Protein quantification showed that TLE+FsCW and MM+FsCW had 45 and 30 fold, respectively, more proteins than TLE+GLU, and this difference was observed in the bidimensional gels, as the two supernatants from media supplemented with FsCW had around 200 spots and the one supplemented with glucose only had 18. TLE+FsCW and MM+FsCW had above 80% of spot similarity. A total of 100 proteins were excised from all three conditions and submitted to mass spectrometry analysis. 85 out of 100 proteins were identified. The only protein observed in all three conditions is a small protein, called epl1, involved in eliciting plant-response against phytopathogens. An aspartic protease, previously described as related to mycoparasitism, was only found when *T. harzianum* was grown with glucose. Gene expression was evaluated and confirmed the gel results. In the media supplemented with FsCW, different hydrolases like chitinases, beta-1,3-glucanases, glucoamylases, alpha-1,3-glucanases, and proteases were identified. Some proteins like a small cystein-rich, alpha-L-arabinofuranosidase and NPP1, with no known function in mycoparasitism were also identified. *T. harzianum* ALL42 is able to inhibit the growth and parasitize *F. solani* and showed a complex and diverse arsenal of proteins that are secreted in response to the presence of the cell walls, with novel proteins not previously described in mycoparasitism studies.

Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers. Carol E. Davis, Howard S. Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521.

Phytophthora infestans is the causative agent of late blight and was responsible for the Irish famine in the 1840's. Today it still continues to be a global problem and in the USA it has been reported that the economic loss on potato crops alone exceeds $6 billion per year. A successful phytopathogenic relationship depends on the ability of the organism to adapt its metabolism during infection on various nutritional substrates (e.g., plant versus tuber) and at different times throughout infection when nutrients may be limiting. Investigation of this metabolic adaptation is key to understanding how *P. infestans* succeeds as a pathogen. To do this, tomato plants and potato tubers were infected with zoospores using a "dipping" method. RNA was extracted at 3 dpi and 6 dpi and subsequently used in library preparation. Following this, the libraries were quality checked by analysis on a Bioanalyzer using a high sensitivity DNA chip. Using Illumina technology (50 bp, paired-end reads) RNA Sequencing was performed. For each sample an average of 262 million reads was obtained. As a reference for the *in planta* data, RNASeq was also performed on defined and complex media. Mining of the data shows that the expression profiles of some pathways change, such as glycolysis and gluconeogenesis. Learning how metabolic adaptation occurs will prove useful in the development of novel control strategies for this plant pathogen.

The fungi strike back: multidrug resistance in Aspergillus fumigatus and agricultural use of fungicides. Paul E. Verweij. Medical Microbiology, UMC St Radboud, Nijmegen, Netherlands.

Aspergillus fumigatus is a saprophytic mould that causes a range of diseases in humans. The spectrum of diseases includes allergic conditions, aspergilloma and acute and chronic invasive aspergillosis. Acute invasive aspergillosis occurs typically in patients with compromised host defenses such as those receiving treatment for leukemia. The management of invasive aspergillosis is very difficult as the diagnostic tools often lack sensitivity and the number of antifungal agents effective against the infection is limited. The azoles are the most important class of agents used for the treatment and prevention of invasive aspergillosis. Since 1998 resistance to medical triazoles has emerged in the Netherlands in clinical A. fumigatus isolates. These isolates commonly showed a multi-azole resistant phenotype and patients with azole-resistant aspergillosis failed to respond to azole therapy. The mortality rate of azole-resistant invasive aspergillosis was 88%. In more than 90% of resistant isolates a combination of changes in the target gene Cyp51A was found: a substitution at codon 98 and a 34 bp tandem repeat in the promoter region (TR34/L98H). As person-to-person transmission is highly unlikely in invasive aspergillosis, the dominance of a single resistance mechanism could not be explained by resistance development in azole-treated patients. Surveys in the environment showed that A. fumigatus isolates resistant to medical triazoles could be recovered from the environment, especially from cultivated soil. The resistance mechanisms in these isolates were identical to those found in clinical isolates. Molecule alignment studies identified 5 triazole fungicides that showed highly similar molecule structures to the medical triazoles. These 5 fungicides have been authorized between 1990 and 1996, thus preceding the isolation of the first resistant clinical A. fumigatus isolate. TR34/L98H is increasingly reported in European countries, India, China and Iran. Recently a new azole-resistance mechanism was found in Dutch patients and in the environment. The resistance mechanism again consisted of a combination of changes in the Cyp51A-gene: TR46/Y121F/T289A. This new resistance mechanism has spread in addition to TR34/L98H in the Netherlands, and has also been reported in the neighboring country Belgium. The selection of multiple azole resistance mechanisms in the environment posses a threat for the use of azole drugs in the management of Aspergillus diseases in humans. Research that investigates the selection of azole resistance in A. fumigatus in the environment is urgently warranted.

Effect of antifungal resistance on virulence of *Candida* spp. L. Vale-Silva, A. Lohberger, D. Sanglard. Inst Microbiology, Univ Lausanne and Univ Hosp Center, Lausanne, VD, Switzerland.

Our laboratory has been involved in the understanding of mechanisms of antifungal resistance in fungal pathogens. While resistance is beneficial to fungi in the presence of antifungals both in vitro and in vivo, resistance mechanisms have some impact on the ability of fungal pathogens to propagate in the host and cause disease. We have addressed this question with two pathogens, *C. albicans* (Ca) and *C. glabrata* (Cg). Our studies have focused on resistance to azoles, which are widely used compounds. In Cg, azole resistance in clinical isolates is almost exclusively mediated by ABC transporters via gain of function (GOF) mutations in the transcription factor (TF) *CgPDR1*. We observed that development of azole resistance in Cg was correlated with gain of virulence and fitness in animal models as compared to susceptible isolates. Recent results suggest that ABC transporters (especially *CgCDR1*) participate to this phenotype. In further studies on interactions of Cg with murine bone marrow-derived macrophages (BMDM), we found that the GOF mutant was able to evade phagocytosis as compared to a matched susceptible isolate. This effect was a consequence of impaired adhesion. This suggests that the mechanism behind escape from phagocytosis was rather based on decreased recognition and/or adhesion by host macrophages. GOF mutations in *CgPDR1* may thus allow Cg to evade the host's innate immune response, which may in turn contribute to increased virulence. In Ca azole resistance is mediated by mutations in TF and in azole targets. GOF mutations in the three different activators including *TAC1*, *MRR1* and *UPC2* regulate specific sub-classes of drug resistance genes belonging to ABC-transporters, major facilitators and azole targets, respectively. We have constructed sets of isogenic strains lacking or carrying each GOF mutations in these TF and addressed their effect on virulence and tissue colonization in animal models. The presence of GOF mutations in *TAC1* and *MRR1* had a neutral effect on virulence and ability to colonize host tissues. A *UPC2* GOF mutation was negatively affecting virulence and tissue colonization, which suggests that *UPC2* activity generates fitness costs in Ca. Our results therefore highlight that the costs of resistance mechanisms on virulence are depending on the fungal species and the type of resistance mechanism.

From enzyme to fungal development or how *sdhB* mutations impact respiration, fungicide resistance and fitness in the grey mold agent *Botrytis cinerea*. Anais Laleve[1], Anne-Sophie Walker[1], Stephanie Gamet[2], Valerie Toquin[2], Daniele Debieu[1], Sabine Fillinger[1]. 1) BIOGER, INRA, Thiverval-Grignon, France; 2) BAYER SAS, Bayer CropScience, Lyon, France.

Respiration inhibitor fungicides are widely used to control fungal diseases on multiple crops. The succinate dehydrogenase inhibitors (SDHIs) are among the latest introduced molecules against the grey mold agent *Botrytis cinerea* on grapevine. We have recently isolated and characterized *B. cinerea* field strains resistant to the SDHIs. Most of the strains harbor one single mutation in the succinate dehydrogenase subunit gene, *sdhB* affecting the ubiquinone-binding pocket. In this study we have introduced these mutations into a *B. cinerea* wild-type strain (B05.10) in order to evaluate the impact of each mutation on SDH- and respiratory activity and inhibition by SDHIs from different chemistries. We also analyzed several parameters of *B. cinerea's* life cycle to assess the fitness cost associated with the resistance mutation. Our results show a strict correlation between the *sdhB* mutation and the resistance spectra to SDHIs. These resistances can be fully explained by the affinities of the SDHIs to its modified target enzyme. Four out of the seven *sdhB* alleles led to significantly reduced SDH activity and, in three cases (H272L, N230I, P225L), to reduced respiration rates. Concerning the fungal biology, we tested mycelial growth and sclerotia production on different media and temperatures, conidia production and germination, resistance to oxidative stress and ROS production, as well as pathogenicity on tomato and bean leaves. All mutants were affected for at least one parameter. However, fitness parameters of mutants $sdhB^{H272R}$ and $sdhB^{P225L}$ showed the strongest modifications among all strains, e.g., reduced pathogenicity, strongly reduced conidia- and sclerotia-production (H272R). A clear correlation between fitness and respiration on one hand, fitness and allele frequency among natural populations on the other, is not yet obvious. However, our results in terms of resistance spectra and fitness parameters should help defining more efficient treatment strategies against grey mold.

Deciphering fungicide resistance mechanisms in phytopatogenic fungi, towards an assessment of resistance risk in new active ingredient research. Gabriel Scalliet[1*], Andreas Mosbach[1], Diana Steinhauer[1], Edel Dominique[1], Robert Dietrich[2]. 1) Disease Control, Syngenta Crop Protection Munchwilen AG, Stein, Switzerland; 2) Syngenta Biotechnology, Inc 3054 Cornwallis Rd. Research Triangle Park, NC 27709.

Resistance to crop protection fungicides can lead to a rapid loss of efficacy in the field. This is a major threat for the sustainability of our products. An early assessment is required in order to define best recommendations in product usage but also guide our choice in developing novel active ingredients. In addition to monitoring the situation for marketed fungicides, we are conducting early assessments for new active ingredients which include resistance generation, mode of resistance identification and fitness penalty determination. High throughput sequencing has become key for a rapid identification of resistance mechanisms both for novel fungicides but also to holder chemistries for which the resistance mechanisms were so far not confirmed. A few examples will be discussed.

Saturday, March 16 2:00 PM–5:00 PM
Merrill Hall

Parallels between Fungal Pathogens of Plants and Animals

Co-chairs: Barbara Howlett and Axel Brakhage

Emerging fungal (and Oomycete) threats to plant and ecosystem health. Sarah J. Gurr[1*], Daniel Bebber[1], Matthew Fisher[2]. 1) Plant Sciences, Oxford University, Oxford, Oxfordshire; 2) Imperial College of Science, Technology and Medicine, London.

Fungal diseases have increased in severity and scale since the mid 20th Century and now pose a serious challenge to global food security and ecosystem health (Gurr et al., 2011 Fungal Biology Reviews 25 181-188). We have demonstrated recently that the threat to plants of fungal infection has now reached a level that outstrips that posed by bacterial and viral diseases combined (Fisher et al., 2012 Nature 484 185-194) I shall highlight some of the more notable fungal and oomycete plant diseases and will draw attention to the emergence of new pathotypes affecting crop yields and decimating our natural and managed landscapes. We have calculated that the losses due to persistent disease (that is, non-epidemic) caused by, for example rice blast, wheat stem rust, corn smut, soybean rust and potato late blight, if mitigated, would be sufficient to feed 8.5% of the global population (based on 2000 calories per day for 1 year). Moreover, tree losses due to fungal and oomycete diseases such as dutch elm, chestnut blight, sudden oak death, jarrah die-back and pine beetle/blue stain fungus, thus far, have been estimated to account for significant CO2 sequestration losses (Fisher et al., 2012). The spread of such organisms around the world is facilitated primarily by trade, but there is increasing concern that climate change may allow their establishment in regions hitherto deemed unsuitable. Increasing latitudinal ranges are anticipated under rising temperatures. However, the interactions between climate change, crops and natural enemies are complex, and the extent to which crop pests and pathogens have altered their latitudinal ranges in response to global warming is largely unknown. We can demonstrate, from thousands of observations of hundreds of pests and pathogens, their shift polewards since 1950, with a more rapid shift since 1990 (Bebber et al., (under review)). This latitudinal shift is seen in both Hemispheres. Moreover, the rate of movement since 1950 is identical to that predicted by global climate data. This observed trend cannot be explained by latitudinal variation in technical capacity to detect and report pest incidences.

Melanin as virulence determinant of human and plant pathogenic fungi. Axel A. Brakhage, Andreas Thywissen, Juliane Macheleidt, Sophia Keller, Vito Valiante, Thorsten Heinekamp. Molec & Appl Microbiology, Leibniz Inst Natural Prod Res Infection Biol-HKI, Jena, Germany.

In fungi, melanins are often associated with the cell wall and also contribute to the structural rigidity of spores. In several plant and human pathogenic fungi, melanins contribute to pathogenicity. For example, pigmentless mutants of the plant pathogens *Magnaporthe oryzae* and *Colletotrichum lagenarium*, as well as the human-pathogenic fungi *Cryptococcus neoformans* and *Aspergillus fumigatus* are less virulent when compared to melanin-producing wild-type strains. In *M. oryzae*, it was shown that a 1,8-dihydroxynaphthalene (DHN) melanin layer between the cell wall and the cell membrane is essential for turgor generation. The melanin acts as a barrier to the efflux of solute from the appressorium, which occurs as pressure is generated. Cellular turgor is translated into mechanical force of infection hyphae, forcing it through the leaf cuticle[(1)]. In human-pathogenic fungi, high turgor pressure is not required for penetration of tissue. In these fungi, melanin displays other virulence attributes such as the scavenging of reactive oxygen species. In *A. fumigatus*, at least two types of melanin are produced: Pyomelanin by polymerization of homogentisic acid, and DHN melanin. Transcription of genes essential for pyomelanin and DHN-melanin biosynthesis is detected during infection of mice. However, pyomelanin seems to be dispensable for fungal virulence in the murine infection models tested[(2,3)]. DHN melanin is responsible for the grey-green color of *A. fumigatus* conidia. The biosynthesis enzymes of DHN melanin are encoded by six genes. Centrally is the polyketide synthase gene *pksP*, whose deletion results in a mutant strain with drastically attenuated virulence. Recent data of our laboratory showed that DHN melanin is essential not only for inhibition of apoptosis of phagocytes by interfering with the host PI3K/Akt signaling cascade but also for effective inhibition of acidification of conidia-containing phagolysosomes[(4,5)]. These features allow *A. fumigatus* to survive in phagocytes and thereby to escape from human immune effector cells and to become an aggressive pathogen. 1) Wilson RA & Talbot NJ (2009) Nat Rev Microbiol. 7: 185-195 2) Keller et al. (2011) PLoS One 6:e26604 3) Schmaler-Ripcke et al. (2009) Appl Environ Microbiol. 75: 493 4) Thywiben et al. (2011) Front Microbiol. 2: 96 5) Volling et al. (2011) Cell Microbiol. 13: 1130.

Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections. Joanna Potrykus[1], David Stead[2], Dagmar S Urgast[3], Donna MacCallum[1], Andrea Raab[3], Jörg Feldmann[3], Alistair JP Brown[1]. 1) Aberdeen Fungal Group, University of Aberdeen, Aberdeen, United Kingdom; 2) Aberdeen Proteomics, University of Aberdeen, Aberdeen, United Kingdom; 3) Trace Element Speciation Laboratory, University of Aberdeen, Aberdeen, United Kingdom.

Iron is a vital micronutrient that can limit the growth and virulence of many microbial pathogens. Here we show, that in the murine model of disseminated candidiasis, the dynamics of iron availability are driven by a complex interplay of localized and systemic events. As the infection progresses in the kidney, *Candida albicans* responds by broadening its repertoire of iron acquisition strategies from non-heme iron (*FTR1*-dependent) to heme-iron acquisition (*HMX1*-dependent), as demonstrated *in situ* by laser capture microdissection, RNA amplification and qRT-PCR. This suggested changes in iron availability in the vicinity of fungus. This was confirmed by ^{56}Fe iron distribution mapping in infected tissues via laser ablation-ICP-MS, which revealed distinct iron exclusion zones around the lesions. These exclusion zones correlated with the immune infiltrates encircling the fungal mass, and were associated with elevated concentrations of murine heme oxygenase (HO-1) circumventing the lesions. Also, MALDI Imaging revealed an increase in heme and hemoglobin alpha levels in the infected tissue, with their distribution roughly corresponding to that of ^{56}Fe. Paradoxically, whilst iron was excluded from lesions, there was a significant increase in the levels of iron in the kidneys of infected animals. This iron appeared tissue bound, was concentrated away from the fungal exclusion zones, and was accompanied by increased levels of ferritin and HO-2. This iron accumulation in the kidney correlated with defects in red pulp macrophage function and red blood cell recycling in the spleen, brought about by the fungal infection. Significantly, this effect could be replicated by selective chemical ablation of splenic red pulp macrophages by clodronate. Collectively, our data indicate that systemic events shape micronutrient availability within local tissue environments during fungal infection. The infection attenuates the functionality of splenic red pulp macrophages leading to elevated renal involvement in systemic iron homeostasis and increased renal iron loading. Simultaneously, localized nutrient immunity limits iron availability around foci of fungal infection in the kidney. In response, the fungus modulates its iron assimilation strategies.

Common strategies in plant and human "necrotrophic" pathogens: role of PCD. N. Shlezinger[1], H. Irme[2], G. Braus[2], A. Sharon[1]. 1) Tel Aviv University, Tel Aviv, Israel; 2) Georg-August University Goettingen, 37077 Goettingen, Germany.

Botrytis cinerea is a model system to study pathogenicity of necrotrophic fungi. As inferred by the term "necrotrophic", such pathogens must first kill host cells before they can use them as a source of nutrients. To achieve this, *B. cinerea* promotes apoptotic cell death in infected plants, which facilitates lesion spreading during late infection stage. How the fungus survives the first encounter with living plant tissue remained unclear. We found that hyphae of B. cinerea undergo massive PCD during early stages of infection, but fully recover upon transition to second phase of infection. Further studies using the fungal and plant mutants showed that survival, and hence pathogenicity of the fungus depended on anti-apoptotic machinery; fungal mutants bearing defects in the anti-apoptotic gene BcBIR1 had reduced virulence whereas strains over-expressing this gene were hypovirulent. A similar phenomenon was observed with another necrotrophic plant pathogen, but not with two hemi-biotrophic pathogens. These results showed that the anti-PCD machinery is essential for pathogenic development necrotrophic plant pathogens. In order to extend these finding to other systems, we generated transgenic strains of the human pathogen *Aspergillus fumigatus*. This fungus shares common stages of infection with B. cinerea and hence might also need the anti-PCD machinery for infection. Indeed, when conidia of A. fumigates are inoculated on a sensitive tissue, the developing hyphae undergo massive cell death, and similar to B. cinerea they fully recover later. Collectively, our results show that certain plant and human fungal pathogen share common strategies and mechanisms when infecting their host. One such mechanism is control of host-induced cell death, through conserved anti-apoptotic machinery.

Septin-mediated plant tissue invasion by the rice blast fungus Magnaporthe oryzae. Yasin Dagdas, Lauren Ryder, Michael Kershaw, Nick J. Talbot. Dept Biological Sci, Univ Exeter, Exeter, United Kingdom.

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious economic problems affecting rice production. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. Development of a functional appressorium requires completion of a single round of mitosis shortly after conidial germination, which also leads to initiation of autophagic recycling of the contents of the fungal spore to the appressorium. We have recently shown that a hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Septins scaffold F-actin, via the ezrin-radixin-moesin (ERM) protein, Tea1, and phosphatidylinositide interactions at the appressorium plasma membrane. The septin ring assembles in a Cdc42 and Chm1-dependent manner and forms a diffusion barrier to localize the Inverse-Bin-Amphiphysin-RVS (I-BAR)-domain protein, Rvs167, and Wiskott-Aldrich Syndrome protein (WASP), Las17 at the point of penetration. This leads to formation of a penetration hyphae that breaches the host cuticle and leads to plant tissue colonization. We present evidence that septin-mediated plant infection is regulated by a specialised NADPH oxidase-tetraspanin complex necessary for control of F-actin dynamics. We also describe the potential operation of a pressure-mediated checkpoint pathway that leads to initial septin assembly and activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.

Components of the urease complex govern virulence of *Fusarium oxysporum* on plant and animal hosts. Katja Schaefer, Elena Pérez-Nadales, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Cordoba, Spain.

In the soilborne pathogen *Fusarium oxysporum*, a mitogen-activated protein kinase (MAPK) cascade homologous to the yeast filamentous growth pathway controls invasive growth and virulence on tomato plants. Full phosphorylation of Fmk1 requires the transmembrane protein Msb2, a member of the family of signalling mucins that have emerged as novel virulence factors in fungal plant pathogens. A yeast two-hybrid screen for proteins interacting with the Msb2 cytoplasmic tail identified UreG, a component of the urease enzymatic complex. UreG belongs to a set of accessory proteins needed to activate Apo- urease, which converts urea to yield ammonia and carbon dioxide. The *F. oxysporum* genome contains two structural urease genes, *ure1* and *ure2*. Mutants in *ureG* or *ure1* showed reduced growth on urea as the sole carbon and nitrogen source. Lack of urease activity in the mutants resulted in failure to secrete ammonia and to increase the extracellular pH. The D*ureG* mutants caused significantly reduced mortality on tomato plants and on the animal model host *Galleria melonella*, while D*ure1* mutants only showed reduced virulence on tomato plants. Real-time qPCR analysis of key genes involved in nitrogen uptake and assimilation, as well as in the urea cycle, during infectious growth of *F. oxysporum* in *G. melonella* revealed increased transcript levels of arginase, which converts arginine to urea. Our results suggest a role for the urease accessory protein UreG in fungal virulence on plant and animal hosts.

The role of LysM effectors in fungal fitness. Anja Kombrink[1], Jason Rudd[2], Dirk-Jan Valkenburg[1], Bart Thomma[1]. 1) Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom.

LysM effector genes are found in the genomes of a wide range of fungal species. The encoded LysM effectors are secreted proteins that contain a varying number of LysM domains, which are carbohydrate-binding modules. Ecp6, secreted by tomato leaf mould fungus Cladosporium fulvum, is the first characterized LysM effector. We demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as a microbial-associated molecular pattern (MAMP) and triggers immune responses upon recognition by the host. Ecp6 outcompetes plant receptors for chitin binding, and thus prevents the activation of immune responses. Many fungal genomes, including saprophytes, carry multiple LysM effector genes that share only low sequence conservation and encode a varying number of LysM domains. We speculate that fungal LysM effectors might bind different carbohydrates and exert various functions in fungal fitness. In the fungal wheat pathogen Mycosphaerella graminicola, two LysM effectors were identified. Mg3LysM, but not Mg1LysM, suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both Mg1LysM and Mg3LysM inhibit degradation of fungal hyphae by plant chitinases, revealing an additional function for LysM effectors in pathogen virulence. We recently observed that Mg1LysM binds to the bacterial cell wall constituent peptidoglycan. Similarly, a LysM effector from the saprophytic fungus Neurospora crassa showed peptidoglycan binding. We hypothesize that peptidoglycan binding by LysM effectors plays a role in the interaction of fungal species with bacterial competitors. The soil-borne fungal pathogen Verticillium dahliae contains seven LysM effectors genes of which one (Vd2LysM) is induced during tomato infection. Inoculation with two independent knock-out mutants revealed that Vd2LysM is required for full virulence of V. dahliae. However, Vd2LysM does not specifically bind chitin and does not function in a similar fashion as previous characterized LysM effectors. Thus, its function in virulence remains unclear.

Genes important for *in vivo* survival of the human pathogen Penicillium marneffei. Harshini C. Weerasinghe, Michael J. Payne, Hayley E. Bugeja, Alex Andrianopoulos. Genetics, The University of Melbourne, Parkville, Victoria, Australia.

Pathogenic fungi are having an increasing global impact in the areas of health, agriculture and the environment. As such it is essential to understand the mechanisms that fungi employ to survive and grow within a host. The emergence of many new "opportunistic fungal pathogens" has to a great extent altered the traditional view that pathogenicity was solely reliant on the inherent properties of the pathogen. In fact, the ability of a pathogen to cause disease in some hosts but not in others suggests that pathogenic determinants are complex and dynamic, and are largely dependent on specific pathogen-host relationships. Despite this there are conserved aspects of the interactions between host and pathogen. For example., hosts employ innate immune responses as an almost immediate recognition and attack mechanism against invading pathogens. Penicillium marneffei is a temperature dependent dimorphic fungus, growing in a hyphal form producing conidia at 25°C and as a yeast form at 37°C. Despite its importance as an opportunistic pathogen, little is known about the biology and mechanism of infection of P. marneffei. The infectious agents (conidia) are believed to be inhaled, reaching the alveoli of the lungs, where they are phagocytosed by alveolar macrophages for elimination. At this point that P. marneffei switches growth to a pathogenic yeast cell form, and is able to withstand macrophage cytotoxic attacks to cause infection. In order to understand how P. marneffei responds to the host, RNA-seq analysis was used to create a transcriptomic profile of P. marneffei, when infected in murine macrophages. These results were compared to RNA-seq data from hyphal (25°C) and yeast (37°C) cells grown *in vitro* in order to identify genes that are specifically upregulated during infection. Based on this analysis a group of genes of varying functions were chosen for gene deletion studies and tested for defects in pathogenicity. Among these is a group of Pep1-like aspartic endopeptidases which are a uniquely expanded family in P. marneffei and that show reduced virulence in a macrophage model.

Saturday, March 16 2:00 PM–5:00 PM
Chapel

Secondary Metabolism

Co-chairs: Gillian Turgeon and Bettina Tudzynski

Genomic profiles of secondary metabolism genes in *Cochliobolus* pathogens. B. Condon[1*], D. Wu[1], K. Bushley[3], I. Grigoriev[2], C. Elliott[4], B. Howlett[4], B. G. Turgeon[1]. 1) Plant Pathology & Plant-Micro Biology, Cornell University, Ithaca, NY; 2) DOE Joint Genome Institute, Walnut Creek, CA; 3) Dept of Plant Pathology, Oregon State University, Corvallis OR; 4) School of Botany, University of Melbourne, Melbourne, AU.

The genomes of five *Cochliobolus heterostrophus* strains, two *Cochliobolus sativus* strains, and three additional *Cochliobolus* species (*Cochliobolus victoriae, Cochliobolus carbonum, Cochliobolus miyabeanus*) were sequenced at the Joint Genome Institute (JGI). These species are notable pathogens of economically important grasses and many produce a signature secondary metabolite host selective toxin, conferring high virulence to a host of a particular genotype. We catalogued the suites of secondary metabolism genes [focusing on those encoding backbone nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs)] in these genomes and performed comparative phylogenomic analyses within the *Cochliobolus* genus and in a kingdom-wide context. We found that NPS and PKS genes were broadly conserved, disparately conserved, or species-unique. Distribution patterns for conserved or disparately conserved genes suggest an evolutionary mechanism involving rapid duplication, loss, and recombination of protein domains. Genes categorized as species unique within our dataset often had lone orthologs in phylogenetically distant species. Expanding genomic resources may reveal that 'signature' genes, formerly thought to be species-unique are present broadly, but sporadically, in other fungi. These results have strong implications for understanding evolution of genes for host selective toxins and associated virulence.

A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, *Leptosphaeria maculans*. Candace Elliott[1], Damien Callahan[2], Daniel Schwenk[3], Markus Nett[4], Dirk Hoffmeister[3], Barbara Howlett[1]. 1) School of Botany, University of Melbourne, Melbourne, Australia; 2) Metabolomics Australia, School of Botany, The University of Melbourne, Victoria 3010, Australia; 3) Friedrich-Schiller-Universität, Department Pharmaceutical Biology at the Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany; 4) Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany.

Phomenoic acid, a long chain aliphatic carboxylic acid, is a major metabolite produced by *Leptosphaeria maculans*, a fungal pathogen of *Brassica napus* (canola). Early biosynthetic studies suggested that the methyl group derived from S-adenosylmethionine (SAM), whereas the incorporation pattern of [13C] acetate suggested a polyketidic origin of the linear portion of phomenoic acid (Devys et al., 1984). We have used domain modelling to predict a candidate polyketide synthase (PKS) for phomenoic acid biosynthesis. Of the 15 predicted polyketide synthases (PKS) in the *L. maculans* genome, seven were reducing with the appropriate domains (KS - keto-synthase; AT - acyltransferase; DH - dehydratase; MT- methyltransferase; ER - enoylreductase; KR - ketoreductase; ACP- acyl carrier protein) for the biosynthesis of phomenoic acid. The most highly expressed of these seven genes, *PKS2*, was silenced to 10% of that of wild type levels and the resultant mutant produced 25 times less phomenoic acid than the wild type did, indicating that *PKS2* is involved in phomenoic acid biosynthesis. This gene is part of a cluster and nearby genes are co-regulated. A two-fold reduction in the expression of the adjacent transcriptional regulator *C6TF*, led to at least a 20-fold reduction in expression of *PKS2*, as well as of other genes in the cluster (*P450*, *YogA*, *RTA1* and *MFS*), but not of the adjacent *ChoK* or a hypothetical gene (*Hyp*). This down-regulated mutant also showed a marked reduction in phomenoic acid production. Phomenoic acid is toxic towards another canola pathogen *Leptosphaeria biglobosa* 'canadensis', but *L. maculans* and to a lesser extent the wheat pathogen, *Stagonospora nodorum* are more tolerant. Phomenoic acid may play a role in allowing *L. maculans* to outcompete other fungi in its environmental niche.

Fusarin C biosynthesis in *Fusarium fujikuroi*: the fusarin C gene cluster, their function and regulation. Eva-Maria Niehaus[1], Karin Kleigrewe[2], Philipp Wiemann[1], Lena Studt[1,2], Hans-Ulrich Humpf[2], Bettina Tudzynski[1]. 1) Institute of Plant Biology and Biotechnology, Schlossplatz 8, 48143 Muenster, Germany; 2) Institute of Food Chemistry, Corrensstr. 45, 48149 Muenster, Germany.

The filamentous fungus *F. fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins, pigments and mycotoxins. In order to reduce the health risk of mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of mycotoxin biosynthesis genes is of great importance. The recently sequenced genome of *F. fujikuroi* contains 17 polyketide synthases (PKS). So far only four of them can be linked to specific products: bikaverin, fusarins, fumonisins and fusarubins. The focus of this work is studying the biosynthesis and regulation of the mutagenic mycotoxin fusarin C by external signals, such as nitrogen availability and pH. Furthermore the involvement of potential transcription factors and global regulators such as AreA, AreB, GS, PacC and three members of the *velvet*-like complex (Vel1, Vel2, Lae1) were investigated. We show that all nine genes are co-expressed under nitrogen sufficient conditions. Chromatin immunoprecipitation (ChIP) experiments revealed a high level of H3K9 acetylation under these favorite conditions. By combination of gene deletion and overexpression of the cluster genes and co-cultivation of different mutants, we were able to identify the intermediates and finally unraveled the entire fusarin biosynthetic pathway which we are presenting in a model.

Cellular development integrating primary and induced secondary metabolism in the filamentous fungus *Fusarium graminearum*. Jon Menke[1], Jakob Weber[2], Karen Broz[3], H. Corby Kistler[1,3*]. 1) Department of Plant Pathology, University of Minnesota, St. Paul, USA; 2) Molekulare Phytopathologie, Universität Hamburg, Germany; 3) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

Several species of the filamentous fungus *Fusarium* colonize plants and produce toxic small molecules that contaminate agricultural products, rendering them unsuitable for consumption. Among the most destructive of these species is *F. graminearum*, which causes disease in wheat and barley and often contaminates the grain with harmful trichothecene mycotoxins. Induction of these secondary metabolites occurs during plant infection or in culture in response to chemical signals. Here we report that trichothecene biosynthesis involves a complex developmental process that includes dynamic changes in cell morphology and the biogenesis of novel subcellular structures. Two cytochrome P-450 oxygenases (Tri4p and Tri1p) involved in early and late steps in trichothecene biosynthesis were tagged with fluorescent proteins and shown to co-localize to vesicles we call “toxisomes.” Toxisomes, the inferred site of trichothecene biosynthesis, dynamically interact with motile vesicles containing a predicted major facilitator superfamily protein (Tri12p) previously implicated in trichothecene export and tolerance. The immediate isoprenoid precursor of trichothecenes is the primary metabolite farnesyl pyrophosphate. When cultures are shifted from non-inducing to trichothecene inducing conditions, changes occur in the localization of the isoprenoid biosynthetic enzyme HMG CoA reductase. Initially localized in the cellular endomembrane system, HMG CoA reductase increasingly is targeted to toxisomes. Metabolic pathways of primary and secondary metabolism thus may be coordinated and co-localized under conditions when trichothecene synthesis occurs.

LaeA sleuthing reveals cryptic gene clusters in pathogenic Aspergilli. Nancy Keller[2], Wenbing Yin[2], Saori Amaike[2], Katharyn Affeld[2], JinWoo Bok[2], Daniel Schwenk[3], Dirk Hoffmeister[3], Joshua Baccile[1], Ry Forseth[1], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA; 2) Department of Plant Pathology, Department of Medical Microbiology and Immunology, and Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA; 3) Department of Pharmaceutical Biology at the Hans-Knöll-Institute, Friedrich-Schiller-Universität, Beutenbergstrabe 11a, 07745 Jena, Germany.

The human and plant pathogenic Aspergilli, Aspergillus fumigatus and A. flavus, are known to produce a plethora of secondary metabolites. However, most of these metabolites are not yet characterized although their gene clusters are apparent from genomic sequence. In both species, the nuclear protein LaeA regulates the expression of many of these uncharacterized gene clusters. Following leads from laeA mutant microarray data, we created gene deletion and overexpression strains and used 2D NMR-based comparative metabolomic analyses to identify previously undescribed metabolites from both species. In A. fumigatus a tryptophan-derived iron(III)-complex, hexadehydro-astechrome (HAS), was found to be the major product of the cryptic has non-ribosomal peptide synthetase (NRPS) cluster. In A. flavus we show that two separate clusters encode enzymes that produce partially overlapping sets of novel piperazines, pyrazines, and morpholines. These L-tyrosine metabolites are activated by two NRPS-like proteins, LnaA and LnbA. Loss and overexpression of these metabolites impacted fungal development in these species.

The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in *Fusarium graminearum*. Kristina M. Smith, Lanelle R. Connolly, Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

The cereal pathogen *Fusarium graminearum* produces secondary metabolites toxic to humans and animals, yet coordinated transcriptional regulation of secondary metabolite gene clusters remains largely a mystery. By ChIP-sequencing we found that regions of the *F. graminearum* genome with secondary metabolite clusters are enriched for a histone modification, trimethylated histone H3 lysine 27 (H3K27me3), associated with gene silencing. This modification was found predominantly in regions that lack synteny with other *Fusarium* species, generally subtelomeric regions. H3K27me3 and di- or trimethylated H3K4 (H3K4me2/3), modifications associated with gene activity, are found in mutually exclusive regions of the genome. To better understand the role of H3K27me3, we deleted the gene for the putative H3K27 methyltransferase, KMT6, a homolog of *Drosophila* Enhancer of zeste, E(z). The *kmt6* mutant lacks H3K27me3, as shown by western blot and ChIP-sequencing, displays growth defects, is sterile, and produces mycotoxins under conditions where they are not generated in wildtype (WT) strains. RNA-sequencing showed that genes modified by H3K27me3 are most often silent, as about 75% of the 4,449 silent genes are enriched for H3K27me3. Surprisingly, we found 22% of the 8,855 expressed genes enriched for H3K27me3. A subset of genes that were enriched for H3K27me3 in WT gained H3K4me2/3 in *kmt6* (1,780 genes), and an overlapping set of genes showed increased expression. Almost 95% of the remaining 2,720 annotated silent genes showed no enrichment for either H3K27me3 or H3K4me2/3. In these cases absence of H3K27me3 is insufficient for expression, which suggests a requirement for additional factors for gene expression. Taken together, we show that absence of H3K27me3 allows expression of 14% of all annotated genes, resulting in derepression of predominantly secondary metabolite pathways and other species-specific functions, including potentially secreted pathogenicity factors. This study provides the framework for novel targeted strategies to control the “cryptic genome” and specifically secondary metabolite expression.

Secondary metabolism in *Botrytis cinerea*: the grey and pink sides of a pathogen. M. Viaud[1], H. Sghyer[1], J. Schumacher[2], A. Simon[1], B. Dalmais[1], J.M. Pradier[1]. 1) INRA, BIOGER, Av. L. Brétignières, 78850 Grignon, France; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47906, USA.

Sequencing the genome of the grey mould fungus *Botrytis cinerea* revealed 40 secondary metabolism (SM) gene clusters corresponding to the biosynthesis of mostly unknown compounds including polyketides (21), terpenes (11) and non-ribosomal peptides (8). The two identified phytotoxic metabolites are the polyketide botcinic acid (BOA) and the sesquiterpene botrydial (BOT). Transcriptomic studies previously identified the corresponding BOA and BOT gene clusters. Deletion of the key enzyme BcBOA6 (PolyKetide Synthase) or BcBOT2 (SesquiTerpene Cyclase) encoding genes did not cause any defects, while *bcboa6/bcbot2* double mutants were significantly impaired in necrotrophic development on several hosts suggesting that the two compounds have a redundant function. We are currently investigating how the BOT and BOA gene clusters are regulated: The BOA cluster contains a gene (*bcboa13*) encoding a C6H6 zinc finger transcription factor (TF). Surprisingly, while *bcboa6* mutant has no virulence defect, *bcboa13* mutant shows a drastic reduced virulence which is even more pronounced than that of the *bcboa6/bcbot2* double mutant lacking both known toxins. A whole transcriptome analysis of *bcboa13* mutant is underway to determine whether other genes apart from those of the BOA cluster are regulated by this TF. In opposite to the BOA cluster, the BOT cluster does not contain any TF-encoding gene. We therefore set up a Yeast-One-Hybrid library that contains the majority of *B. cinerea* TFs (393 out of 406) and screened it with the promoter of *bcbot2*. This led to the identification of a C2H2 TF called BcYOH1. Inactivation of *bcyoh1* gene and expression analysis revealed that this TF acts as a global regulator of SM, regulating the expression of genes of the BOT, BOA, and 20 other SM clusters. As in other fungi, the *Velvet* complex takes part in regulation of light-dependent development and SM in *B. cinerea*. Gene inactivation of *bcvel1*, however, does not significantly modify the production of BOA and BOT. Instead, BcVEL1 plays a significant role in the regulation of oxalic acid (OA) formation and pigmentation: it regulates negatively the synthesis of melanin and positively the synthesis of OA and bikaverin (BIK) another polyketidic pigment that is only produced in rare pink strains of *B. cinerea*.

Is fungal secondary metabolism regulated by competing insects? Annika Regulin[1], Nancy Keller[2], Frank Kempken[1]. 1) Department of Botany, Christian-Albrechts University, Kiel, Germany; 2) Department Medical Microbiology and Immunology, Dept of Bacteriology, UW-Madison, USA.

Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxin) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4). In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. The vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with Drosophila larvae microarray analysis was performed. This led to the identification of secondary metabolite genes up- or down-regulated under these conditions. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression at different time points. Fungal single, double and triple mutations of identified up-regulated genes are currently analyzed in confrontation assays to identify potential modifications in gene expression and the survival rate of larvae concerning to chemical defense reaction of fungus-insect interaction compared to wild type. This could reveal insights about the biological function of secondary metabolite genes and clusters such as *stc* and *mdp*.

(1.) Rohlfs, M., Albert, M., Keller, N. P., and Kempken, F. (2007) Biol Lett 3, 523-25. (2.) Kempken, F., and Rohlfs, M. (2010) Fungal Ecol 3, 107-14. (3.) Rohlfs, M., Trienens, M., Fohgrub, U., and Kempken, F. (2009) in "The Mycota XV. (Anke, T., Ed.), Springer Heidelberg, New York, Tokyo, pp. 131-51 (4.) Kempken, F. (2011) Mol Ecol 20, 2876-77.

Saturday, March 16 2:00 PM–5:00 PM
Heather

Light Sensing and Circadian Rhythms

Co-chairs: Luis Larrondo and Reinhard Fischer

Circadian rhythms in gene expression in Aspergillus nidulans. Maria Olmedo[1,2], Julio Rodriguez-Romero[3,4], Julian Röhrig[3], Martha W. Merrow[1,2], Reinhard Fischer[3]. 1) Institute for Medical Psychology, Ludwig-Maximilians-Universität-München, München, Bavaria, Germany; 2) Department of Molecular Chronobiology, University of Groningen, The Netherlands; 3) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Germany; 4) Centre for Plant Biotechnology and Genomics Universidad Politécnica de Madrid(UPM) Campus de Montegancedo Autopista M-40 (Km 38) 28223-Pozuelo de Alarcón (Madrid).

The circadian clock is an endogenous timekeeper that allows organisms to predict cyclic changes in their environment derived from the rotational movement of the Earth. The fungus Neurospora crassa has substantially contributed to the understanding of the mechanism of the circadian clock, mainly due to the presence of an easily assayable rhythm in conidiation. Our understanding of circadian rhythms tells us that they are present in most organisms (from bacteria to humans), yet they have been difficult to detect in other fungal species, likely due to the absence of a clear readout. To circumvent this problem, we have adopted a tool developed for the study of the clock in Neurospora and other model organism, namely bioluminescent reporter proteins, to study circadian rhythms in Aspergillus nidulans, where a previous report has suggested a circadian clock (Greene et al. 2003). The characterization of the Aspergillus clock represents a further step in the understanding of the biology of this fungal genus, which is of great importance for medical, industrial and agricultural reasons. We have produced strains with the promoters of Neurospora clock controlled gene homologs conJ, ccgA and gpdA fused to the (A. nidulans optimized) firefly luciferase ORF. We observe an oscillation in the bioluminescence signal in these strains in constant conditions, implying the presence of an endogenous oscillator. This free running rhythm is detectable in both constant light and darkness and is entrained by environmental signals. Using these reporters we are studying the contribution of the wc (white collar) homologs and other photoreceptors to the Aspergillus clock. The protein WC-1 is a central molecule of the best-studied Neurospora oscillator, which also comprises the protein FRQ (FREQUENCY). The absence of a FRQ homolog in Aspergillus implies that the characterization of its clockwork may unveil new components of additional Neurospora oscillators.

Circadian clock-gated cell division cycles in *Neurospora crassa*. C. Hong[1], J. Zamborszky[1], M. Baek[1], K. Ju[1], H. Lee[1], L. Larrondo[2], A. Goity[2], A. Csikasz-Nagy[3]. 1) Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH; 2) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile; 3) Randall Division of Cell and Molecular Biophysics, and Institute for Mathematical and Molecular Biomedicine, King's College London, London, SE1 UL, UK.

Asynchronous nuclear divisions are readily observed in filamentous fungi such as *Ashbya gossypii* and *Neurospora crassa*. Our computational simulations, however, predict synchronous circadian clock-gated mitotic divisions if the division cycles of such multinucleated organisms are coupled with circadian rhythms. Based on this hypothesis, we investigate the coupling between the cell cycle and the circadian clock in *Neurospora crassa*. First, we show WC-1-dependent light-induced expression of *stk-29* mRNA (homolog of *wee1*), which suggests that there exists a conserved coupling between the clock and the cell cycle via STK-29 in Neurospora as in mammals. Second, we demonstrate that G1 and G2 cyclins, CLN-1 and CLB-1, respectively, show circadian oscillations with luciferase bioluminescence reporters. Moreover, *clb-1* and *stk-29* gene expressions show circadian clock-dependent light-induced phase shifts, which may alter the timing of divisions. Third, we show circadian clock-dependent synchronized nuclear divisions by tracking nuclear morphology with histone *hH1*-GFP reporter. Synchronized divisions occur late in the evening, and they are abolished in the absence of circadian rhythms (frq^{KO}). Our findings demonstrate the importance of circadian rhythms for synchronized mitotic cycles and establish *Neurospora crassa* as an ideal model system to investigate mechanisms that couple the cell cycle and the circadian clock.

Light regulates growth, stress resistance and metabolism in the fungal pathogen *Aspergillus fumigatus*. Kevin K. Fuller, Carol S. Ringleberg, Jennifer J. Loros, Jay C. Dunlap. Genetics, Geisel School of Medicine at Darmouth, Hanover, NH.

Light serves as an important environmental cue that influences developmental and metabolic pathways in a variety of fungi. Interestingly, orthologs of a conserved blue light receptor, WC-1, promote virulence in two divergently related pathogenic species, *Cryptococcus neoformans* and *Fusarium oxysporum*, suggesting that photosensory systems may be conservatively linked to fungal pathogenesis. *Aspergillus fumigatus* is the predominant mold pathogen of immunocompromised patients, but if and how the organism responds to light has not been described. In this report, we demonstrate that the fungus can indeed sense and distinctly respond to both blue and red portions of the visible spectrum. Included in the *A. fumigatus* photoresponse is a reduction in conidial germination kinetics, increased hyphal pigmentation, enhanced resistance to acute ultra-violet and oxidative stresses, and an increased susceptibility to cell wall perturbation. Through gene deletion analysis we have found that the WC-1 ortholog, LreA, is a bone fide blue light receptor in *A. fumigatus* that is required for the photopigmentation response. However, the *DlreA* mutant retains several blue light mediated responses, including the germination and stress resistance phenotypes , suggesting other blue light receptors are operative in this fungus. We also show that the putative red light sensing phytochrome, FphA, is involved with some, but not all, blue light specific phenotypes, indicating a complex interaction between red and blue light photosystems in *A. fumigatus*. Finally, whole genome microarray analysis has revealed that *A. fumigatus* displays broad patterns of gene induction and repression upon exposure to light. Affected genes are largely metabolic and include those involved in lipid and sterol synthesis, respiration, carbohydrate catabolism, amino acid metabolism and metal ion homeostasis. Taken together, these data demonstrate the importance of the photic environment on the physiology of *A. fumigatus* and provide a foundation for future studies into an unexplored area of this important pathogen.

Shedding light on Botrytis biology: characterization of the WC1 photoreceptor and FRQ homologues in the necrotrophic plant pathogen *Botrytis cinerea*. Paulo Canessa[1], Montserrat Hevia[1], Julia Schumacher[2], Paul Tudzynski[3], Luis F Larrondo[1]. 1) Depto. Genética Molecular y Microbiologia, Pontificia Universidad Catolica de Chile, Santiago, Chile; 2) Purdue University, USA; 3) West F Wilhelms Univ Muenster Germany.

The non-pathogenic fungus *Neurospora crassa* has been a premier model for the study of circadian clocks, but it is the only fungus where clocks have been molecularly characterized. In Neurospora the circadian oscillator is based on the transcriptional control of *frq* expression by the White Collar complex (WCC) composed of WC-1, a blue-light photoreceptor and WC-2, its functional partner. The WCC activates *frq* expression until FRQ promotes the phosphorylation of the WCC, shutting down its transcriptional activity. Then FRQ is progressively phosphorylated and degraded setting the bases of circadian rhythmicity. While circadian clocks modulate defense in plants, little is known about its participation in fungal pathogenicity. In an effort to characterize light responses and the circadian clock in *B. cinerea*, we have generated KO of homologues of *wc-1* and *frq* (*bcwcl1* and *bcfrql*). Our results indicate that light can modulate *B. cinerea* growth and activates transcriptional responses. Most -but not all of them- are mediated by BcWCL1. Moreover, the obtained *bcwcl1* KO mutant strains exhibit enhanced conidiation patterns and susceptibility to hydrogen peroxide. Virulence assays show significantly reduced lesion formation under constant light conditions (LL) and light:dark cycles (LD), but not under constant darkness (DD). Further analysis of the *bcwcl1* KO strains indicates non-altered ROS patterns *in planta* but delayed penetration on onion epidermis. RT-qPCR experiments have confirmed daily oscillations in *bcfrql* levels under LD and DD conditions. Using a BcFRQL-luciferase translational reporter, we have further observed oscillatory levels of the BcFRQL protein under both temperature cycles and constant culture conditions. Both BcFRQL and *bcfrql* respond to light pulses, while under oscillatory culture conditions, BcFRQL anticipates cyclical-environmental changes, a key characteristic of circadian behavior. Finally, *bcfrql* KO mutants also exhibit impaired pathogenicity, with a drastic reduction in spore production. To our knowledge, these results provide the first evidence of functional light/circadian machineries in *B. cinerea*, showing that in this necrotroph, homologues of circadian clock components modulate the plant pathogen interaction from a fungal perspective. Fondecyt 1090513, postdoc 3110127, AT24121100, ICGEB CRPCHI0902.

The transcription factor FL is phosphorylated and interacts with a trehalose related protein in *Neurospora crassa*. Carmen Ruger-Herreros[1], Gencer Sancar[2], Michael Brunner[2], Luis M. Corrochano[1]. 1) Departamento de Genetica, Universidad de Sevilla, Spain; 2) BZH, Universität Heidelberg, Germany.

Several environmental cues, including light, promote a developmental transition in *Neurospora crassa* that leads to the formation of conidia. Conidiation is controlled by FLUFFY (FL), a zinc finger transcription factor. Light activates the transcription of *fl* through the transient binding of the WC complex to the *fl* promoter. Light also activates the transcription of several conidiation genes in *Aspergillus nidulans*, and their *Neurospora* homologs have been identified in the *Neurospora* genome. We have assayed the activation by light of the *Neurospora* homologs of *A. nidulans* conidiation genes (*flbA, flbC, flbD, medA* and *stuA*), and the *Neurospora* conidiation gene *con-10* as a control. Unlike *con-10*, none of the *Neurospora* homologs of the *A. nidulans* conidiation genes were induced by light in vegetative mycelia. However, we found that deletion *fl* resulted in light-dependent mRNA accumulation for all the conidiation genes. This result indicated that the absence of FL allows the binding of the WC complex to the promoter of these genes to activate transcription in a light-dependent manner. We have assayed the amount of WC proteins in the D*fl* and wild type strains but we did not find any difference between the two strains. We expect to identify additional genes deregulated by the absence of FL after massive sequencing of total RNA (RNAseq) using a D*fl* strain and wild-type strain in dark and light conditions. We have investigated the role of FL during conidiation in *Neurospora* using a tagged version of FL. FL is present in vegetative mycelia but the amount increses after light exposure. We observed several forms of FL due to phosphorylation, and and we have determined by mass spectrometry that FL is phosphorylated in several residues. We have immunoprecipitated FL to identify proteins that may interact with FL. We have found a protein that interacts with FL in different growth conditions. This protein has been described in other organisms and plays a role in the ability to grow in the presence of trehalose. Since FL is a transcription factor, we have use FL::3XFLAG strain to do ChIPseq in order to identify the putative binding sites of FL to the DNA. We expect that the results from these experiments will help us to understand in more detail the role of FL in the activation of gene transcription during development.

Regulation of gene expression in response to light in *Trichoderma atroviride*. Jose Cetz[1], Nohemi Carreras-Villaseñor[1], Monica Garcia-Esquivel[1], Ulises Esquivel-Naranjo[2], Jose M. Villalobos-Escobedo[1], Cei Abreu-Goodger[1], Alfredo H. Herrera-Estrella[1]. 1) National Laboratory of Genomics for Biodiversity, CINVESTAV-IPN, Irapuato, Irapuato, Mexico; 2) Facultad de Ciencias Naturales. Universidad Autónoma de Queretaro. Queretaro, Mexico.

Trichoderma is used as a photomorphogenetic model due to its ability to conidiate upon exposure to light. In total darkness, *T. atroviride* grows indefinitely as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. Photoconidiation in *Trichoderma* is controlled by the *blr1* and *blr2*, orthologs of the *N. crassa* white-collar genes. We applied high-throughput sequencing technology to RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white or blue-light, as well as from a photoreceptor mutant (D*blr-1*) exposed to white light. We identified 331 transcripts regulated by white light and 204 responsive specifically to blue light, both induced and repressed, the majority of them *blr1* dependent. Among the genes identified there is a set of transcription factors. We have obtained gene disruption mutants of several of the transcription factors, and all potential light receptors. Using this strategy we have obtained mutants that do not conidiate in response to light, as well as mutants that do not require this stimulus to conidiate, and a conection between light and carbon metabolism. Further, transcriptional analysis in RNAi machinery mutants indicated that light induced conidiation is defective in the D*dcr2*, D*dcr1*D*dcr2*, D*rdr3* and D*ago2* mutants, and significant differences were found in the set of light responsive genes between the dicer mutants and the wild type. These data indicate that in *T. atroviride*, the RNAi machinery plays an important role in controlling development.

Genome-wide analysis of light responses in *Mucor circinelloides*. Victoriano Garre, Sergio López-García, Eusebio Navarro, Santiago Torres-Martínez. Departamento de Genética y Microbiología, Universidad de Murcia, 30100 Murcia, Spain.

Light regulates developmental and physiological processes in a wide range of fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The zygomycete *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available. *Mucor*, like other Zygomycetes, has three *white collar-1* genes (*mcwc-1a, mcwc-1b* and *mcwc-1c*) that code for proteins which present characteristics of photoreceptors. Each *mcwc-1* gene controls a specific response to light. Thus, *mcwc-1a* and *mcwc-1c* control phototropism and photocarotenogenesis, respectively, whereas the *mcwc-1b* function in regulation by light has not been proved. In order to deepen in the regulation by light in *Mucor*, a systematic approach using microarrays was followed to characterize white light-inducible transcriptional changes in wild-type and knockout mutants for each *mcwc-1* gene. Analysis of microarray data revealed that light is mainly a positive signal for transcription in *Mucor*, as in other fungi, since 123 genes were up-regulated in the wild-type strain in response to light, whereas only 26 were down-regulated, considering a threshold of threefold change. Genes strongly induced by light included genes known to be up-regulated by light, like the carotenogenic gene *carB* (74-fold), cryptochrome (45-fold) and *mcwc-1c* (22-fold), supporting reliability of the microarray data. Although many of up-regulated genes code for proteins implicated in protection against light-induced damage, several of them code for protein involved in signal transduction that could be involved in light responses like phototropism. Transcriptomic analysis of *mcwc-1* mutants showed that induction of around 60% of the genes is mediated by *mcwc-1a*, whereas only 1% is mediated by *mcwc-1c* and none is mediated by *mcwc-1b*, suggesting that *mcwc-1a* is the main photoreceptor. Searching for cis-acting regulatory motifs upstream of genes regulated by *mcwc-1a* identified consensus sequences similar to those found in light regulated genes of *Neurospora crassa*. Moreover, the identification of a small group of genes regulated by the three *mcwc-1* genes points out that the three proteins form complexes to regulate gene expression. Funded by MINECO (BFU2012-32246), Spain.

Shedding light on secondary metabolite cluster gene expression, sporulation, UV-damage repair and carotenogenesis in the rice pathogen *Fusarium fujikuroi*. Phillipp Wiemann, Bettina Tudzynski. Institut für Biologie und Biotechnologie der Pflanzen Westfälische Wilhelms-Universität Münster Schlossplatz 8 48143 Münster Germany.

The rice pathogen *Fusarium fujikuroi* produces economically important secondary metabolites like gibberellic acids and carotenoids as well as mycotoxins like bikaverin and fusarin C. Their production is activated in response to environmental stimuli such as light, pH or nutrient availability. In this study, we evaluate the effects of light and different putative light receptors on growth and differentiation as well as secondary metabolism. Bimolecular fluorescence complementation proved that homologs of the *Neurospora crassa* White Collar proteins in *F. fujikuroi* (WcoA and WcoB) form a nuclear localized complex (WCC) that is needed for full functionality. Deletion and complementation of both genes revealed that the WCC represses bikaverin gene expression in constant light conditions and induces immediate light-dependent carotenoid gene expression as shown by northern blot analyses. Additionally the WCC represses conidiogenesis in response to light. The effects observed regarding bikaverin and carotenoid gene expressions as well as conidiogenesis are antagonistically to the ones observed in the velvet mutant, making a connection between the WCC and the *velvet* complex feasible, similarly to the situation in *Aspergillus nidulans*. Since carotenoid production was maintained in both *wcoA* and *wcoB* single as well as in *wcoA/B* double mutants in constant light conditions, we focused on characterization of additional putative light receptors in *F. fujikuroi*. Deletion of the phytochrome-like-encoding gene *fph1* did not show any significant phenotype. Deletion of *phl1*, coding for a cryptochrome/photolyase demonstrated impaired carotenoid biosynthesis gene expression upon exposure to light. Additionally, gene expression and HPLC analyses of these mutants demonstrated loss of fusarin C gene expression and concomitant production formation compared to the wild type, suggesting a distinct transcriptional activity for this barely characterized class of enzymes. Finally UV mutagenesis experiments and qRT-PCR demonstrate that WcoA, WcoB and Phl1 are involved in UV-damage repair most likely by transcriptionally activating *phr1*, encoding a CPD-photolyase. The data presented here allow us to draw a first model of how light receptors function in a signaling network in the rice pathogen *F. fujikuroi*.

Saturday, March 16 2:00 PM–5:00 PM
Fred Farr Forum

Fungal Evo-Devo

Co-chairs: Steve Harris and Brian Shaw

The Molecular Foundations of the Fungal Lifestyle. Antonis Rokas. Department of Biological Sciences, Vanderbilt University, Nashville, TN.

A defining characteristic of Fungi is that, in contrast to plants and animals, they are typically embedded in their food and digest it externally in the presence of competitors (think of the blue lines of mold in blue cheese). Thus, different fungi specialize in "eating" different foods (hence their diverse primary metabolism), and because digestion happens externally, fungi have also evolved potent food defense mechanisms (hence their diverse secondary or specialized metabolism). In many ways, the genes involved in fungal primary metabolism are their "teeth", whereas the genes involved in secondary metabolism are their "horns", "spines" and "claws". I will use examples from our recent studies on fungal human pathogens, domesticated fungi and fungal genome evolution to argue that metabolism and more generally physiology is fundamental to the fungal lifestyle. In contrast to plants and animals, in which most phenotypic evolution proceeds largely through developmental changes affecting growth and morphological form, phenotypic evolution in the fungal kingdom occurs largely through changes in metabolism.

Gene expression and regulation during conidial morphogenesis in *Neurospora crassa*. Daniel J. Ebbole, Shengli Ding, Dawoon Chung, Heather Wilkinson, Shaw Brian. Plant Pathology & Microbiol, Texas A&M Univ, College Station, TX.

The regulatory pathway controlling conidiation in *N. crassa* consists of five genes, *acon-4* (aconidiate), *fl* (fluffy), *acon-3*, *csp-1* (conidial separation) and *csp-2*. The first three genes are required for the transition of aerial hyphae from filamentous growth to the budding pattern resulting in proconidial chain formation. Maturation of the proconidial chain to individual conidia relies on the activation of genes for two additional transcription factors, *csp-1* and *csp-2* that are required for proper interconidial septum formation and spore release. High throughput mRNA sequencing defined a set of genes induced during conidiation and allowed us to identify genes for cell wall degrading enzymes required for conidial separation. Based on expression patterns and epistasis among the regulators, we defined the order of gene action required for conidial morphogenesis from the initiation of budding to the release of mature conidia.

Comparative developmental morphology in lentinoid mushrooms: toward a new fungal evo-devo? David S. Hibbett. Biology, Clark University, Worcester, MA.

Fungi produce a mind-boggling diversity of complex forms, including mushrooms, puffballs, coral fungi, and others. Reconstructing patterns of morphological transformations has been a major focus of fungal molecular systematics, and developmental morphology has been a traditional source of characters for fungal taxonomy. However, few studies have explicitly compared developmental programs in a phylogenetic context, and research into the genetic bases of morphological evolution in fungi has lagged far behind that in plants and animals. In this talk, I will review three cases of naturally occurring developmental variation in the polyphyletic "lentinoid" fungi, which may suggest profitable avenues for studies in fungal evo-devo: 1) Normally agaricoid species of Lentinus, Neolentinus and Lentinellus may be induced to produce coralloid forms under conditions of light deprivation or low temperatures. These phenomena provide examples of phenotypic plasticity that may reflect evolvability of developmental programs. 2) Panus rudis has a short lateral stipe and fruits directly on wood, while the closely related P. fulvus has a dramatically elongated stipe and often fruits from sclerotia immersed in leaf litter. The developmental transformation between forms suggests a case of hypermorphosis, with a delayed onset of pileus initiation in P. fulvus. Light-induced formation of pilei in P. rudis may provide clues to the mechanisms of offset in stipe elongation. 3) Lentinus tigrinus is a gilled mushroom that has a naturally occurring "secotoid" mutant that has an enclosed, puffball-like hymenophore. The secotioid morphology appears to be conferred by a recessive allele at a single locus. Resolving the gene(s) responsible for the secotioid phenotype may provide clues to the evolution of gasteromycetes. Complete genome sequences have been (or are being) produced for species of Lentinus, Panus, Lentinellus and Neolentinus, which will provide opportunities to study the mechanisms underlying inter- and intraspecific developmental variants in lentinoid mushrooms.

The Cdc42 GTPase module and the evolution of conidiophore architecture in *Aspergillus*. Steven D. Harris. Center Plant Sci Innovation, Univ Nebraska, Lincoln, NE.

Conidiophores are reproductive structures that enable filamentous fungi to produce and disseminate large numbers of asexual spores. The diversity in conidiophore morphology is sufficiently large to serve as a basis for fungal systematics. *Aspergillus* and *Penicillium* species are members of the family Trichocomaceae that form conidiophores with characteristic architecture. Whereas the *Penicillium* conidiophore appears to be a modified branched hyphal structure, the *Aspergillus* conidiophore is seemingly more complex and includes additional cell types. Here, it is proposed that the "aspergillioid" conidiophore may have evolved from a "penicillioid" ancestor via changes in expression of key regulators of the GTPases Cdc42 and RacA. In particular, mutations that affect these regulators in *A. nidulans* dramatically alter conidiophore morphology by eliminating terminal vesicles and permitting formation of branched stalks. Because the transcriptional regulatory network that controls conidiophore development in *Aspergillus* is well characterized, further study of how this network links to regulators of Cdc42 should provide fundamental insight into the evolution of developmental morphogenesis in fungi (i.e., fungal evo-devo).

Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase. Audrey M.V. Ah-Fong, Howard S. Judelson. Plant Pathology & Microbiology, University of California, Riverside, CA.

The dual-specificity phosphatase Cdc14 is best known as a regulator of cell cycle events such as mitosis and cytokinesis in yeast and animal cells. However, the diversity of processes affected by Cdc14 in different eukaryotes raises the question of whether its cell cycle functions are truly conserved between species. Analyzing Cdc14 in *Phytophthora infestans* should provide further insight into the role of Cdc14 since this organism does not exhibit a classical cell cycle. Prior study in this organism already revealed novel features of its Cdc14. For example, instead of being post-translationally regulated like its fungal and metazoan relatives, PiCdc14 appears to be mainly under transcriptional control. It is absent in vegetative hyphae where mitosis occurs

and expressed only during the spore stages of the life cycle which are mitotically quiescent, in contrast to other systems where it is expressed constitutively. Since transformants overexpressing PiCdc14 exhibit normal nuclear behavior, the protein likely does not play a critical role in mitotic progression although PiCdc14 is known to complement a yeast Cdc14 mutation that normally arrests mitosis. Further investigation into the role of PiCdc14 uncovered a novel role. Subcellular localization studies based on fusions with fluorescent tags showed that PiCdc14 first appeared in nuclei during early sporulation. During the development of biflagellated zoospores from sporangia, PiCdc14 transits to basal bodies, which are the sites from which flagella develop. A connection between Cdc14 and flagella is also supported by their phylogenetic distribution, suggesting an ancestral role of Cdc14 in basal bodies and/or flagellated cells. To help unravel the link between PiCdc14 and the flagella apparatus, searches for its interacting partners using both yeast two hybrid and affinity purification are underway. Together with colocalization studies involving known basal body/centrosome markers such as centrin and gamma-tubulin, the location and hence the likely roles of PiCdc14 will be revealed.

Molecular Determinants of Sporulation in *Ashbya gossypii*. Jurgen W. Wendland, Lisa Wasserstroem, Klaus Lengeler, Andrea Walther. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Kopenhagen V, Denmark.

Previously we have analysed the pheromone response MAPK signal transduction cascade in *A. gossypii*. The major findings were (i) deletion of both pheromone receptor genes *STE2* and *STE3* did not inhibit sporulation whereas (ii) deletion of the transcription factor *STE12* resulted in hypersporulation (Wendland *et al*. 2011). Here we present our analysis of key *A. gossypii* homologs of *Saccharomyces cerevisiae* sporulation specific genes. We show that mutants in *IME1, IME2, KAR4*, and *NDT80* are blocked in sporulation. Mutants in *IME4, KAR4*, and *UME6* also confer a vegetative growth defect. *IME4* expression was found during vegetative growth while *IME2* was not detected under these conditions. We performed transcriptional profiling of non-sporulating strains and determined a core set of about 50 down-regulated sporulation specific genes in these mutants. Interestingly, this set of down-regulated genes is upregulated in the *A. gossypii ste12* mutant providing regulatory evidence of the hypersporulation phenotype of this mutant. Other genes identified in the RNAseq data indicated that during development of sporangia metabolic genes for nutrient uptake are active. Therefore we performed Return-To-Growth assays with mutants inhibited in the sporulation pathway. These strains were kept under conditions in which the wild type initiates sporulation. This lead to induction of sporangium formation, a stage at which these strains remained. Supply of new nutrients resulted in hyphal outgrowth in all mutants indicating that after initiation of the sporulation program *A. gossypii* can reverted to vegetative growth at different stages. In addition we identified differential regulation of two endoglucanases encoded by *ENG1* and *ENG2*. While *ENG1* was not differentially regulated, *ENG2* was down-regulated in e.g. ime1 but strongly up-regulated in *ste12*. Deletion analysis of *ENG2* showed that Eng2 is required for hyphal fragmentation into individual sporangia. We can thus provide a detailed overview of the genetic regulation of sporulation in *A. gossypii*. A comparison with *S. cerevisiae* highlights the role of *KAR4* in sporulation upstream of *IME1*. Finally, our study provides further evidence that the pheromone signaling response MAPK-cascade in *A. gossypii* has a regulatory control function over sporulation alongside regulation of sporulation by nutritional cues.

THE *velvet* regulators in *Aspergilli*. Heesoo Park, JJae-Hyuk Yu. Bacteriology, University of Wisconsin Madison, Madison, WI.

The *velvet* regulators are the key players coordinating fungal growth, differentiation and secondary metabolism in response to various internal and external cues. All *velvet* family proteins contain the conserved *velvet* homology motif (~190 a.a.), and define a novel class of fungal specific transcription factors with the DNA binding ability. Some *velvet* regulators form time and/or cell type specific complexes with other *velvet* regulators or non-*velvet* proteins. These complexes play differential roles in regulating growth, development, sporogenesis and toxigenesis. Among the *velvet* complexes, the VelB-VosA hetero-complex acts as a functional unit conferring the completion of sporogenesis (focal trehalose biogenesis and spores wall completion), and the long-term viability of spore, and the attenuation of conidial germination in the model filamentous fungus *Aspergillus nidulans*. Both *velB* and *vosA* are activated by AbaA in developing cells, and the VelB-VosA complex plays a dual role in activating genes associated with spore maturation and in exerting negative feedback regulation of developmental genes. Interestingly, the VelB-VosA complex plays similar yet somewhat distinct roles in spore maturation, dormancy and germination in *Aspergillus fumigatus* and *Aspergillus flavus*. A comprehensive model depicting the roles of the velvet regulators in aspergilli is presented.

A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*. J. Aït-Benkhali[1,2], E. Coppin[1,2], S. Brun[1,2,3], T. Martin[4], C. Dixelius[4], R. Debuchy[1,2]. 1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) UFR des Sciences du Vivant, Université Paris-7 Diderot, Paris, France; 4) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden.

High-mobility group B proteins are eukaryotic DNA-binding proteins characterized by the HMG-box functional motif. These transcription factors play a pivotal role in global genomic functions and in the control of genes involved in specific developmental or metabolic pathways. The filamentous ascomycete *Podospora anserina* contains 12 HMG-box genes. Of these, four have been previously characterized; three are mating-type genes that control fertilization and development of the fruiting-body, whereas the last one encodes a factor involved in mitochondrial DNA stability. Systematic deletion analysis of the eight remaining uncharacterized HMG-box genes indicated that none were essential for viability, but that seven were involved in the sexual cycle. Two HMG-box transcription factors display striking features. Pa_1_13940, an ortholog of SpSte11 from *Schizosaccharomyces pombe*, is a pivotal activator of mating-type genes in *P. anserina*, whereas Pa_7_7190 is a repressor of several phenomena specific to the stationary phase, most notably hyphal anastomoses. Constitutive expression of mating-type genes in a *DPa_1_13940* strain did not restore fertility, indicating that *Pa_1_13940* has additional functions related to sexual reproduction besides activating mating-type genes. RT-qPCR analyses of HMG-box genes in different HMG-box deletion strains indicated that *Pa_1_13940* is at the hub of a network of several HMG-box factors that regulate the sexual cycle. Complementation experiments with a strain deleted for mating-type genes revealed that this network control fertility genes in addition to mating-type target genes. This study points to the critical role of the HMG-box members in sexual reproduction in fungi, as 11 out of 12 members were involved in the sexual cycle in *P. anserina*. Pa_1_13940 and SpSte11 are conserved transcriptional regulators of mating-type genes, although *P. anserina* and *S. pombe* have diverged 1.1 billion years ago. Two HMG-box genes, *SOX9* and its upstream regulator *SRY*, play also an important role in sex determination in mammals. The mating-type genes and their upstream regulatory factor form a module of HMG-box genes similar to the *SRY/SOX9* module, suggesting it may be ancestral in Opisthokonta.

Saturday, March 16 2:00 PM–5:00 PM
Kiln

Environmental Metagenomics

Co-chairs: Chris Schadt and Betsy Arnold

Microbial Responses to a Changing Climate: Implications for the Future Functioning of Terrestrial Ecosystems. Donald R. Zak. University of Michigan, Ann Arbor, MI.

Soil harbors a phylogenetically diverse community of microorganisms whose physiological activity mediates the biogeochemical cycling of carbon and nitrogen at local, regional, and global scales. These microbial communities are structured by the physical environment as well as the availability of growth-limiting resources (i.e., organic compounds in plant detritus). Presently, human activity is manipulating both the physical conditions and the availability of limiting resources to soil microbial communities at a global scale, but the implications of doing so for the future functioning of ecosystems is presently unclear. In this presentation, I will discuss the ways in which humans are manipulating the ecological constraints on microbial communities in soil, the compositional and functional responses that may result, and identify gaps in our knowledge that limit our ability to anticipate the response of microbial communities and ecosystem processes in a changing environment. Using an array of metagenomic approaches, I will provide evidence that rates of atmospheric N deposition expected in the near future can down regulate the transcription of fungal genes with lignocellulolytic function, thereby altering microbial community composition, slowing plant litter decay, and increasing soil C storage. This mechanism is not portrayed by any biogeochemical model simulating ecosystem response to atmospheric N deposition, and it demonstrates that microbial communites in soil may respond to a changing environment in ways that have unanticipated consequences for the future functioning of terrestrial ecosystems.

The Interaction of *Mycoplasma*-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host. Mizue Naito[1], Teresa Pawlowska[2]. 1) Dept. of Microbiology, Cornell University, Ithaca, NY; 2) Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY.

Arbuscular mycorrhizal fungi (AMF), comprising the monophyletic phylum Glomeromycota, are obligate biotrophs, and form symbiotic associations with 80% of terrestrial plants. AMF associate symbiotically with the roots of plants, and are specialized in the transfer of nutrients from the soil to the plant host. In return for increased nutrient uptake, the plants supply AMF with up to 20% of their photosynthetically derived carbohydrates. Thus, AMF symbiosis contributes significantly to global nutrient cycling and terrestrial ecosystems. AMF have been known to harbour two types of bacteria in their cytoplasm: (i) the *Burkholderia*-related *Candidatus* Glomeribacter gigasporarum and (ii) a *Mycoplasma*-related bacteria, which we refer to as *Mycoplasma*-related endobacteria (MRE). MRE live freely in the AMF cytoplasm, and have been found associated with all lineages of AMF worldwide. Virtually nothing is known about the MRE, such as their evolution, biological capabilities, and whether they are mutualists or parasites of their AMF hosts. In order to understand the nature of this symbiosis, and determine the role that the MRE play in arbuscular mycorrhizae, next generation sequencing (Roche 454 and Illumina) was performed on MRE isolated from 3 distinct AMF hosts, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Racocetra verrucosa*. Phylogenetic reconstruction and divergence dating using 22 conserved genes have revealed that MRE form a novel monophyletic subclade of the *Mycoplasmas* and have diverged from their *Mycoplasma* relatives at least 400 million years ago, which may indicate the establishment of the MRE-AMF association to be quite ancient. Analysis of annotated genes have revealed novel proteins that are likely to play a role in interacting directly with the fungal host. Preliminary data suggest that MRE are important in enabling the completion of the life cycle of their AMF hosts.

Metagenomic analysis reveals hidden fungal diversity in grass rhizosphere and tree foliage. Ning Zhang[1], Stephen Miller[1], Shuang Zhao[1], Hayato Masuya[2]. 1) Plant Biology and Pathology, Rutgers Univ, New Brunswick, NJ; 2) Dept Forest Microbiology, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, JAPAN.

The diversity of microorganisms on earth remains poorly understood. Unculturable fungi inhabiting rhizosphere, phyllosphere, and other less studied niches are thought to represent a large fraction of the unknown diversity. In this study, we used both culture-dependent method and Illumina metagenomic sequencing approach to explore fungal diversity in two environments: grass (*Poa pratensis*, Kentucky bluegrass) rhizosphere and tree (*Cornus* spp., dogwood) foliage. For the grass rhizosphere sample, Illumina metagenomic analysis identified 1,192 fungal genera from 20.8 million reads, while the culture-based method identified 21 genera. For the *Cornus* sample, metagenomic analysis identified 73 fungal genera from 6.6 million reads, while 22 genera were isolated from culture. From both cases, we found that metagenomic sequencing analysis revealed significantly higher fungal diversity than culture-based method, which will help us better understand the diversity and role of fungi in the ecosystem.

Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus. Weiguo Fang, Xiaoxuan Chen. College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China.

Inspite being of great concern to human health and the management of plants and animals, the mechanisms facilitating host switching of eukaryotic pathogens remain largely unknown. The endophytic insect-pathogenic fungus Metarhizium robertsii evolved directly from endophytes and its entomopathogenicity is an evolutionarily acquired characteristic. We found that M.robertsii acquired a sterol carrier (Mr-NPC2a) from an insect by horizontal gene transfer (HGT). Mr-NPC2a increased the amount of ergosterol in hyphal bodies by capturing sterol from insect hemolymph, and thus maintained cell membrane integrity and improved fungal survival rate. On the other hand, the reduction in sterol (substrate for molting hormone synthesis) in insect hemolymph elongated larval stage, which allows the fungus to fully exploit host tissues and produce more conidia. This is first report of HGT from host to a eukaryotic pathogen, and the host gene ultimately improved the infectivity of the pathogen.

Structure and function of soil fungal communities across North American pine forests. Kabir Peay[1], Jennifer Talbot[1], Dylan Smith[1], Rytas Vilgalys[2], John Taylor[3], Thomas Bruns[3]. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Biology, Duke University, Durham, NC; 3) Plant & Microbial Biology, UC Berkeley, Berkeley, CA.

Fungi are a critical component of the diversity and function of terrestrial ecosystems. They regulate decomposition rates, facilitate plant nutrient uptake and have a profound impact on agriculture and economics. Understanding the forces that structure fungal communities thus has important theoretical and

practical implications. While ecologists have long recognized the importance of scale on ecological processes, fungal communities have primarily been studied at small-scales, focusing on deterministic processes. To rectify this knowledge gap we are using next generation sequencing techniques to survey soil fungi in North American pine forests with a sampling design that allows us to examine community structure from roots-to-biomes. Our results show that soil fungal communities in these ecosystems are highly diverse and are structured primarily by large-scale, macroecological processes, rather than small-scale deterministic processes. Our results also show that there is high functional redundancy in soil fungal communities. This work demonstrates that increasing the scale of observation is critical to a complete understanding of the ecological dynamics of soil fungi and the ecosystem processes they mediate.

Genomic analysis of Mortierella elongata and its endosymbiotic bacterium. Gregory Bonito[1], Andrii Gryganskyi[1], Christopher Schadt[2], Dale Pelletier[2], Amy Schaefer[3], Gerald Tuskan[2], Jessy Labbé[2], Sofia Robb[4], Rebecca Ortega[1], Francis Martin[5], Mitchel Doktycz[2], Kurt LaButti[6], Matt Nolan[6], Robin Ohm[6], Igor Grigoriev[6], Rytas Vilgalys[1]. 1) Duke University, Durham NC; 2) Oak Ridge National Laboratory, Oak Ridge TN; 3) University of Washington, Seattle WA; 4) University of California, Riverside CA; 5) Institut National de la Recherche Agronomique, Nancy France; 6) Joint Genome Institute, Walnut Creek CA.

Mortierella belong to a group of basal fungi (Mortierellomycotina) common to soils and the rhizosphere and endosphere of many plant species. Mortierella species are known for rapid growth and abundant lipid production. Mortierella elongata is one species commonly isolated from forest soils and healthy plant roots where it grows asymptomatically as an endosymbiont. Mortierella elongata is a heterothallic species but can also reproduce asexually through chlamydospores and sporangiospores. Recent reports indicate that some isolates of M. elongata host endosymbiotic bacteria, which may be transmitted vertically via spores. However, it is still unclear whether all Mortierella species host endosymbionts or whether these are lineage-specific associations. Given the geographically widespread distribution of Mortierella elongata and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont to address whether there are genomic signatures of co-adaptation or co-evolution in the genomes of Mortierella and its endosymbiotic bacterium, which may impact the function and growth of Mortierella elongata. The 50 Mb genome of M. elongata was sequenced to 112x coverage. Of the 220,113 putative proteins identified in M. elongata, 109,093 appear to be unique (e.g. only ~50% have orthologs in other fungal species having sequenced genomes). The M. elongata genome appears to be enriched in genes related to tryptophan metabolism, siderophore group nonribosomal peptides, glucan 1,4-alpha glucosidases, and in lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophopholids) compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the M. elongata isolate is related to Glomeribacter (endosymbiont of Gigospora, Scutellospora, and other Glomeromycota) within the Burkholdariales. The ~2.6 MB endosymbiont genome is larger than that of Glomeribacter but quite reduced compared to free-living isolates of Burkholdaria. The reduced genome size of this bacterium, and the fact that it has thus far evaded pure culture isolation, supports the view that this is an ancient and obligate symbiosis.

Integrative genomics of poplar-fungal pathogen interactions. Richard C. Hamelin. Forest Sciences, University of British Columbia, Vancouver, BC, Canada.

Poplar is an important tree, both from an ecosystemic point of view as riparian species, and as a commercial agro-forestry crop for the production of wood and paper products and increasingly as a source of bioenergy. Fungi in the Urediniales and the Dothideomycetes are responsible for the most important diseases of poplars. In most tree-fungal pathogen interactions, a few founder species are key determinants of the outcome. To better understand these interactions at the landscape level and predict their outcome, we are using a variety of genome-based approaches. We have applied DNA barcoding and multigene phylogeny to poplar pathogens to assess species diversity and host specificity both from environmental and herbarium-derived samples. We have found a high level of fidelity in host tracking of pathogens vis-à-vis their host. However, this fidelity tends to break down when poplar is grown in intensive plantations or when interspecific hybrids are planted. To further investigate and understand this pattern, we sequenced the genomes of 12 poplar pathogens with different host specificity. By comparing these genomes we identified core gene sets as well as genes that are unique to each species and are candidate determinants of the interaction outcomes. Annotation of these genome sequences with customized pipelines allowed us to assign putative functions to the candidate genes and detect effector-like sequence profiles. RNAseq profiling of the interaction of poplar rust on different hosts and their hybrids confirms the uniqueness of expression patterns in host-specific infections. In addition, we are using population genome re-sequencing approaches to detect selection patterns at the genome level. Several of the candidate genes identified in the upstream analyses encode secreted proteins and possess a signature of positive selection. Integration of this data can be used as predictive resources to determine the outcome of poplar-pathogen interactions in the environment.

Fungal pathogen and endophyte genetics within the context of forest community dynamics. M.-S. Benitez[1], M. H. Hersh[2], L. Becker[1], R. Vilgalys[3], J. S. Clark[1,3]. 1) Nicholas School of the Environment, Duke University, Durham, NC; 2) Department of Biology, Eastern Michigan University, Ypsilanti, MI; 3) Department of Biology, Duke University, Durham, NC.

Fungal pathogens play important roles in forest community dynamics, particularly through negative-density dependent regulation. Negative-density dependence regulation is hypothesized to be regulated by the presence of host-specific pathogens. Studies on forest pathogens, however, indicate the predominance of generalist seedling pathogens, capable of infecting more than one host species. To understand the mechanisms through which "generalist" pathogens contribute to forest-community dynamics we conducted extensive surveys of seedling pathogens in temperate hardwood forests of the eastern U.S.A. Species in the genera *Colletotrichum* and *Ilyonectria* were among the most commonly isolated and recovered amplicon sequence from seedlings of multiple host species showing disease symptoms. Further, co-infection by both *Colletotrichum* and *Ilyonectria* species decreases host survival, as quantified by posterior model probabilities. To investigate molecular mechanisms associated with multi-host generalism and co-infection, and to determine whether these "generalist" pathogens are distinct species or species-complexes, the genomes of three common species in our dataset (e.g. *C. fioriniae*, *C. gloesporoides* and *Ilyonectria europea*) were sequenced. The largest genome of the three belonged to *Ilyonectria* at 63.66 Mb, which also contained the highest number (22,250) of genes. The smallest genome belonged to *C. fioriniae* with 50.04 Mb and 15,777 genes. Genome size and number of predicted genes appears expanded, confirming their role as seedling pathogens. For instance, three out of four polysaccharide lyase (PL) enzyme domains found in fungal genomes, are enriched in these three species. PL enzymes are relevant in plant pathogenicity since they may contribute to initial stages of host penetration. The genome sequence of these fungal groups will serve as a reference set for population level studies to address host-specificity and local adaptation within our isolate database.

Saturday, March 16 2:00 PM–5:00 PM
Nautilus

Dimorphic Transitions

Co-chairs: Anne Dranginis and Alex Andrianopoulos

Epigenetic Switching Regulates the Yeast-Hyphal Transition in *Candida albicans*. Haoyu Si[1], Allison Porman[1], Matthew Hirakawa[1], Stephen Jones[1], Aaron Hernday[2], Alexander Johnson[2], Richard Bennett[1]. 1) Mol Microbiol & Immunology, Brown University, Providence, RI; 2) Mol Microbiol & Immunology, UCSF, San Francisco, CA.

Candida albicans is a dimorphic yeast that is normally found as a commensal organism in the mammalian gastrointestinal tract. It is also a prevalent opportunistic pathogen able to infect multiple mucosal and internal sites in the human body. A principle feature of *C. albicans* biology is its ability to grow in multiple phenotypic states, including both yeast and filamentous forms. Phenotypic plasticity is also exemplified by the "white-opaque switch", in which cells can reversibly transition between the white and opaque states. White and opaque forms differ in multiple aspects including their shape, their interaction with host immune cells, their mating competency, and their pathogenesis. Furthermore, white cells are induced to form hyphal filaments when grown at 37°C, neutral pH, or in the presence of serum, whereas opaque cells do not form filaments in response to these conditions, and this difference could explain the decreased virulence of opaque cells in models of systemic infection. In this study, we show that opaque cells can undergo the yeast-filament transition in response to environmental cues, but that these cues are distinct from those that induce the transition in white cells. For example, growth on low phosphate medium or medium containing the sugar sorbitol induced efficient filamentous growth in opaque cells, while these conditions did not induce filamentous growth in white cells. Genetic dissection of the regulation of opaque cell filamentation showed extensive overlap with the regulation of filamentation in white cells, including roles for the established transcriptional regulators Ume6, Efg1, and Tup1. However, genes induced by filamentous growth in opaque cells showed only limited overlap with those induced during white cell filamentation. Together, these studies indicate that *C. albicans* white and opaque cells are both capable of undergoing filamentation but do so in response to different environmental signals and generate distinct transcriptional profiles, reflecting intrinsic differences in the programming of the two phenotypic states.

Extracellular and intracellular signaling orchestrates morphotype-transition and virulence in human pathogen *Cryptococcus neoformans*. Linqi Wang, Xiuyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, College Station, TX.

Interactions with the environment and divergent species drive the evolution of microbes. To sense and rapidly respond to these dynamic interactions, "simple" microbes developed bet-hedging social behaviors, including the construction of heterogeneous biofilm communities and transition between different morphotypes. The human fungal pathogen *Cryptococcus neoformans* can undergo morphotype transition between the yeast and the filamentous form. Most recently, we demonstrated that the zinc-finger regulator Znf2 bridges the bi-direction yeast-hypha transition and virulence in this pathogen. One of Znf2 downstream targets is extracellular protein Cfl1. Cfl1 is a cell-wall bound adhesin and a signaling molecule when it is released. This matrix protein Cfl1 plays a similar but less prominent role than Znf2 in orchestrating morphogenesis and virulence in *C. neoformans*. Through transcriptome analyses and screening Znf2 downstream targets by overexpression, we identified an additional player in the control of morphogenesis and biofilm formation. This factor is an intracellular RNA-binding protein Pum1. As expected, Pum1 affects filamentation in a Znf2 dependent manner. However, the effect of Pum1 on morphogenesis is independent of Cfl1. The *pum1*D *cfl1*D double mutant shows a more severe defect in filamentation than either of the single mutant, indicating that Pum1 and Cfl1 act in two parallel pathways. Two of Pum1's targets, Fad1 and Fad2, form a *Cryptococcus*-specific adhesin family. Like Cfl1, these two extracellular adhesins show regulatory roles in conducting morphogenesis and virulence in *C. neoformans* and thus may be involved in extracellular signaling transduction. Our results indicate that complex regulatory cascades composed of extracellular and intracellular circuits may be responsible for mediating morphological transition in response to the cues in the environments and the host.

***Histoplasma* strain variations and differences in pathogenic-phase transcriptomes.** Jessica A. Edwards[1], Chenxi Chen[2], Megan M. Kemski[1], Thomas K. Mitchell[2], Chad A. Rappleye[1]. 1) Microbiology, Ohio State University, Columbus, OH; 2) Plant Pathology, Ohio State University, Columbus, OH.

The morphological dimorphism of *Histoplasma capsulatum* reflects an underlying change in gene expression that is essential for pathogenesis. In the yeast-phase, *Histoplasma* infects the mammalian lung and proliferates within phagocytic cells. Geographically distinct strains of *Histoplasma* exhibit differences in their relative virulence and in their pathogenic mechanisms. The close similarity in the genome sequences of these diverse strains suggests that phenotypic variations result from gene expression differences rather than gene content. To better understand how the transcriptional program translates into morphological and pathogenic differences between strains, we profiled the yeast-phase transcriptomes of two *Histoplasma* strains by RNAseq methodology. For both strains, about 50% of sequence reads align to the genome providing evidence for approximately 9000 genes. Quantitative comparisons reveal about 200 genes are at least 10-fold differentially expressed between strains, and these include genes related to *Histoplasma* pathogenesis (*SOD3*, *YPS3*, *AGS1*). The genes encoding the secreted calcium-binding protein (*CBP1*), histone proteins (*H2B*, *H3*, and *H4*) and an ammonium transporter are among the most highly expressed genes overall. Using GFP-transcriptional fusions and their introduction into both strain backgrounds, we demonstrate that dissimilarity in the transcriptional activity of individual genes reflects variations in the trans-acting factors between strains rather than the sequence of the promoters, themselves. These studies lay an essential foundation to facilitate discovery of the factors that contribute to strain-specific virulence differences of *Histoplasma*.

The C_2H_2 transcription factor HgrA promotes hyphal growth in the dimorphic pathogen *Penicillium marneffei*. Hayley E. Bugeja, Michael J. Hynes, Alex Andrianopoulos. Department of Genetics, University of Melbourne, Parkville, VIC, Australia.

Penicillium marneffei (recently renamed *Talaromyces marneffei*) is well placed as a model experimental system for investigating fungal growth processes and their contribution to pathogenicity. An opportunistic pathogen of humans, *P. marneffei* is a dimorphic fungus that displays multicellular hyphal growth and asexual development (conidiation) in the environment at 25°C and unicellular fission yeast growth in macrophages at 37°C. We have characterised the transcription factor *hgrA* (hyphal growth regulator), which contains a C_2H_2 DNA binding domain closely related to that of the stress-response regulators Msn2/4 of *Saccharomyces cerevisiae*. HgrA is not required for controlling yeast growth in response to the host environment, nor does it appear to have a key role in response to stress agents, but is both necessary and sufficient to drive the hyphal growth program. *hgrA* expression is specific to hyphal growth and its deletion affects multiple aspects of hyphal morphogenesis and the dimorphic transition from yeast cells to hyphae. Loss of HgrA also causes cell wall defects, reduced expression of cell wall biosynthetic enzymes and increased sensitivity to cell wall, oxidative, but not osmotic stress agents. As well as causing apical hyperbranching during hyphal growth, overexpression of *hgrA* prevents conidiation and yeast growth, even in the presence of inductive cues. HgrA is a strong inducer of hyphal growth and its activity must be appropriately regulated to allow alternative developmental programs to occur in this dimorphic pathogen.

A conserved splicing factor is required for vesicle transport in *Ustilago maydis*. Nikola Kellner[1], Kai Heimel[1,2], Florian Finkernagel[3], Theresa Obhof[1], Joerg T. Kaemper[1]. 1) Dept. of Genetics, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Dept. of Molecular Microbiology and Genetics, Georg-August-University Göttingen, Göttingen, Germany; 3) Institute for Molecular Biology and Tumor Research, Marburg, Germany.

In the corn smut fungus *Ustilago maydis*, sexual development is initiated by the fusion of two yeast-like haploid sporidia, resulting in a filamentous dikaryon that is capable to infect the plant. Growth as a dikaryon requires an elaborate coordination of the cell cycle, the migration and distribution of the nuclei and polar hyphal growth. We have identified the *U. maydis* Num1 protein with a pivotal function during these processes. Num1 is homologous to SPF27, a core component of the evolutionary conserved Prp19/CDC5 complex (NTC). The NTC contributes to splicing efficiency and fidelity, but is also involved in cell cycle checkpoint control, response to DNA damage or formation and export of mRNP-particles. Deletion of num1 in *U. maydis* has no obvious phenotype in sporidia, however, hyphae exhibit polarity defects; in addition, the *num1* mutation affects the cell cycle and cell division. We identified Cdc5 and Prp19, two conserved components of the Prp19/CDC5 complex, as Num1 interactors. In line with the function of the NTC, we demonstrated by means of a genome-wide mRNA-Seq analysis that splicing in *num1* deletion strains is impaired on a global level. In addition to the NTC components, several proteins with putative functions during vesicle-mediated transport processes were identified as Num1 interactors; in particular the conventional kinesin 1 motor protein Kin1 was shown to physically interact with Num1. Both *num1*- and *kin1*-deletion strains exhibit identical phenotypes with respect to filamentous and polar apical growth, the morphology of vacuoles, the subcellular distribution of the Dynein motor protein as well as the motility of early endosomes, strongly corroborating a genetic interaction between Num1 and Kin1. Our data implicate a previously unidentified connection between a component of the splicing machinery and cytoplasmic transport processes. As the *num1* mutation also affects cytoplasmic mRNA transport, the protein might constitute a novel functional interconnection between the two disparate processes of splicing and trafficking.

***N*-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi.** Sarah A. Gilmore[1], Shamoon Naseem[2], James B. Konopka[2], Anita Sil[1]. 1) Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY.

Cellular differentiation is an essential process for the development and growth of multicellular eukaryotic organisms. Similarly, many unicellular organisms undergo a program of cellular differentiation to produce a new cell type specialized for survival in a distinct environmental niche. Systemic dimorphic fungal pathogens, such as *Histoplasma capsulatum* (*Hc*) and *Blastomyces dermatitidis* (*Bd*), can switch between a unicellular parasitic yeast form adapted for growth within mammals and an infectious soil-growing filamentous form as part of their natural life cycles. Temperature is thought to be the predominant environmental cue that promotes cellular differentiation of systemic dimorphic fungi; however, work with other fungi indicates that additional environmental cues including CO2, light, and nutrient availability can influence how an organism responds to its environment. Recent work suggests that the ubiquitous monosaccharide *N*-acetylglucosamine (GlcNAc) can play a role in cell signaling in fungi. We identified GlcNAc as a potent inducer of the yeast-to-filament transition in *Hc* and *Bd*. Micromolar concentrations of exogenous GlcNAc were sufficient to induce a robust morphological transition of *Hc* yeast cells to filamentous cells at room temperature, indicating that dimorphic fungal cells may be sensing GlcNAc, or one of its catabolic byproducts, to promote filamentation. Using GlcNAc as a tool to induce a robust and more synchronous phase transition of *Hc* yeast cells to filaments, we examined the temporal regulation of the *Hc* transcriptome during morphogenesis to reveal candidate genes involved in establishing the filamentous growth program. Two genes we identified during transcriptome analysis included *NGT1* and *NGT2*, which encode GlcNAc major facilitator superfamily transporters. RNAi depletion of *NGT1* or *NGT2* rendered *Hc* cells unable to respond to exogenous GlcNAc. Furthermore, wild type levels of *NGT1* and *NGT2* transcripts were important for efficient *Hc* yeast-to-filament conversion even in the absence of exogenously added GlcNAc. These data suggest that Ngt1 and Ngt2 may monitor endogenous GlcNAc as part of an autoregulatory system that allows *Hc* to regulate its filamentous growth.

A GATA transcription factor encoded by *SREB* functions as a global regulator of transcription in *Blastomyces dermatitidi*. Amber Marty, Aimee T. Broman, Christina Kendziorski Gregory M. Gauthier. University of Wisconsin - Madison, 1550 Linden Drive, Microbial Sciences Building, Madison, WI, 53706.

The thermally dimorphic fungi infect several million people each year including those with normal immune defenses. These fungi grow as mold in the soil (22°C) where they produce infectious conidia. Upon soil disruption, aerosolized conidia are inhaled into the lungs (37°C) where they convert into yeast. This reversible, temperature-dependent phase transition defines the lifestyle of the dimorphic fungi. In *Blastomyces dermatitidis*, we discovered *SREB* (siderophore biosynthesis repressor in *Blastomyces*), which encodes a GATA transcription factor that promotes the conversion to mold at 22°C and also regulates iron homeostasis. To begin to dissect how *SREB* affects the transcriptional response to temperature, we used gene expression microarrays and chromatin immunoprecipitation with quantitative real-time PCR (ChIP-qPCR). For microarrays, RNA was isolated from *SREB*Δ and wild type (WT) isolates at baseline (yeast) and 6, 24, and 48-hours after a drop in temperature to 22°C. LIMMA and EBarrays were used to identify differentially expressed (DE) genes. For ChIP, we engineered SREB to contain an in-frame, C-terminal 3x-HA tag. SREB-3xHA was cross-linked to its DNA binding targets *in vivo* using 1% formaldehyde at 37°C and 48-hours at 22°C. Following chromatin shearing and reversal of cross-links, enrichment for SREB-3xHA binding (vs. isogenic control) at GATA motifs was assessed by qPCR. Gene expression microarray analyses indicated that *SREB* was a global regulator of transcription at 37°C and 22°C. Gene Ontology enrichment demonstrated *SREB* was involved with diverse processes including iron ion binding, amino acid transport, metabolic

process, and fatty acid biosynthesis. Complementary analysis using weighted gene co-expression network analysis identified modules with DE genes enriched for transmembrane transport, metabolic process, transcription factor activity, translation, and fatty acid biosynthesis. To identify candidate genes for ChIP-qPCR, we integrated gene expression microarray data with genome-wide *in silico* analysis of GATA transcription factor binding motifs. Using this approach, we identified a subset of genes bound and regulated by SREB including *SIDA, MIRB, WD, HAPX,* and *PDH* at 37°C and 22°C. In contrast, enrichment for SREB-3xHA binding of *ECI1* occurred at 22°C, but not 37°C. In conclusion, integration of microarray analysis, *in silico* GATA motifs, GO enrichment, and ChIP-qPCR indicates that *SREB* affects pleiotropic events in *B. dermatitidis*.

Functional Analysis of Genes in Regions of Introgression in Coccidioides. Bridget M. Barker. Immunology & Infectious Diseases, Montana State Univ, Bozeman, MT.

Coccidioides immitis and C. posadasii are dimorphic fungi endemic to the Americas. Genomic analysis of sequenced strains of C. posadasii and C. immitis reveals insights into the population biology of these organisms. There is strong evidence for hybridization and introgression, such that for many of the C. immitis strains, there are several regions that have a closer match to C. posadasii, but few regions within C. posadasii matching C. immitis. Multiple hybridization regions were located in several genomes analyzed, and at least one region containing ten genes exhibits a pattern consistent with introgression in C. immitis. This conserved region was further evaluated in a larger collection of isolates. Approximately half of the C. immitis isolates contain the C. posadasii fragment, and the majority of those are from the southern California and Mexico populations. The region of introgression represents a unique opportunity to functionally assess genes that are likely to be relevant for species-specific virulence and adaptation to mammalian hosts or the environment. This region has a shared recombination point flanking a metalloproteinase, Mep4; genes that are highly expressed in the parasitic phase; and genes of unknown function. Importantly, evolutionary selection has preserved this region in multiple strains of C. immitis further emphasizing the possible role in virulence of these genes. Variation among strains for virulence in murine models of coccidioidomycosis has been observed, but has not been tested in the context of the newly discovered species or with a targeted underlying genetic mechanism hypothesis to test. Gene deletion mutants are being generated for three genes in the conserved introgression region to determine effects on in vitro growth and morphological change under host relevant conditions.

Saturday, March 16 2:00 PM–5:00 PM
Scripps

Tropic Growth and Fusion

Co-chairs: Andre Fleissner and Nick Read

Role of the cell fusion gene *idcA* in fungal mutualism. Carla J. Eaton[1,2], Cornelia Staerkel[1], Barry Scott[1,2]. 1) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand; 2) Bio-Protection Research Centre, Massey University, Palmerston North, New Zealand.

Maintenance of the mutualistic association between the fungal endophyte *Epichloë festucae* and perennial ryegrass relies on a number of important signalling pathways including ROS signalling by the NADPH oxidase (Nox) complex, and the cell integrity- and stress-activated MAP kinase pathways. Perturbation of these signalling pathways leads to a dramatic switch from mutualistic to pathogenic-like association with perennial ryegrass. Interestingly, all *E. festucae* 'symbiotic regulator' genes identified to date are involved in vegetative cell fusion in other fungi, suggesting hyphal fusion may play an important role in maintenance of the mutualistic association. To investigate this putative link, the role of the cell fusion gene *IDC1* (*ham-5*) was examined. *IDC1* is of particular interest as in addition to its role in cell fusion it is also linked to Nox signalling in *Podospora anserina*. Disruption of *E. festucae idcA* leads to a dramatic symbiotic switch from mutualistic to pathogenic-like association with perennial ryegrass. Infected plants are severely stunted and display precocious senescence. Biomass of the D*idcA* mutant *in planta* is significantly increased relative to the wild-type strain, and hyphae extensively colonise host vascular tissues. Formation of intra-hyphal hyphae by the D*idcA* mutant *in planta* is also abundant, possibly due to defective hyphal fusion or defects in septation. The importance of *idcA* for maintenance of mutualistic association with perennial ryegrass supports the hypothesis that hyphal fusion is required for establishment of an interconnected hyphal network essential for mutualism.

Role of extracellular calcium in budding yeast cell fusion. Pablo S. Aguilar. Cell Membranes Laboratory, Institut Pasteur de Montevideo, Montevideo, Uruguay.

The molecular details of membrane fusion during yeast mating are poorly understood. The tetraspanner protein Prm1 is one of the few known components that acts at the step of bilayer fusion. In its absence, mutant mating pairs lyse or arrest in the mating reaction with tightly apposed plasma membranes. The absence of another tetraspanner, Fig1p, which controls pheromone-induced Ca^{2+} influx, yields similar cell fusion defects. Although extracellular Ca^{2+} is not required for efficient cell fusion of wild-type cells, cell fusion in prm1 mutant mating pairs is dramatically reduced when Ca^{2+} is removed. A genetic screen was conducted to uncover genes that promote mating-dependent lysis in the absence of extracelular Ca^{2+}. The role of different candidates in relation to Prm1p will be reviewed here.

The role of calcium and calmodulin during cell fusion and colony initiation in *Neurospora crassa*. Chia-Chen Chang, Nick Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH.

Calcium is an ubiquitous signalling molecule which regulates many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, circadian rhythms, and virulence. Transient increases in cytosolic free calcium ($[Ca^{2+}]_c$) act as intracellular signals. As the primary intracellular Ca^{2+} receptor, calmodulin (CaM) converts these Ca^{2+} signals into responses by regulating the activity of numerous target proteins. We have found that both Ca^{2+}-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit a form of cell fusion called conidial anastomosis tube (CAT) fusion that occurs during colony initiation in the fungal model *Neurospora crassa*. GFP labelled CaM localized as dynamic particles associated with the plasma membrane and moved around within the cytoplasm in both germ tubes and CATs. In particular, CaM showed a dynamic accumulation at two growing tips of CATs that exhibit chemoattraction towards each other. CaM also localized at developing septa in germ tubes. The b-tubulin inhibitor, benomyl, reduced the movement of CaM in the cytoplasm. Moreover, the absence of extracellular Ca^{2+} inhibited the recruitment of CaM to CAT tips as well as inhibiting CAT chemoattraction. The deletion of the *myosin-5* (*myo-5*) gene caused the mis-localization of CaM in tips of growing germ tube and CATs. This suggests that the movement of cytoplasmic CaM involves transport along microtubules, and the recruitment of CaM to tips involves myosin-5 along F-actin and is dependent on extracellular Ca^{2+}.

LFD-1 is a component of the membrane merger machinery during cell-cell fusion in *Neurospora crassa*. Javier Palma-Guerrero, N. Louise Glass. Plant and Microbial Biology Department, UC Berkeley, Berkeley, CA.

Cell-cell fusion is an essential part of the development of most eukaryotic organisms, playing an important role both during sexual development and vegetative growth. In the filamentous fungus *Neurospora crassa*, cell fusion events occur during all stages of the life cycle. This, together with the sequenced and annotated genome and the genetic tools available, makes this fungus a good model system for dissecting cell fusion. Plasma membrane merger is the last step in the cell-cell fusion process, occurring after cell wall remodeling, and it is the definitive event that allows for cytoplasm mixing between the fusing cells. Although molecular mechanisms associated with intracellular membrane fusion are well characterized, the molecular mechanisms of plasma membrane merger between cells are poorly understood. Only one gene encoding a protein involved in this last step of cell fusion had been previously identified in *N. crassa*: *Prm-1*, a deletion of which results in strains that show a »50% reduction in vegetative and sexual cell fusion. We have identified a second gene, *lfd-1*, which is also involved in plasma membrane merger in *N. crassa*. LFD-1 is a plasma membrane protein only present in ascomycete filamentous fungi. *N. crassa* strains carrying a deletion of *lfd-1* results in a reduction in both vegetative and sexual cell fusion, and having a similar, but less severe, phenotype than a *Prm-1* deletion strain. Strains carrying both *Prm-1* and *lfd-1* deletions indicate that LFD-1 acts independently of PRM-1. Strains carrying *Prm-1* or *lfd-1* mutations result in increased cell lysis during cell-cell fusion, a phenotype that was enhanced by reducing extracellular calcium concentration. These results suggest that the lysis phenotype associated with cell fusion events is due to membrane damage caused by defects during membrane merger, and which may be repaired in a calcium dependent process. Our results indicate that both PRM-1 and LFD-1 are important, but non-essential components of the cell fusion membrane merger machinery.

Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in *Neurospora crassa*. Martin Weichert[1], Ewald Priegnitz[1], Raphael Brandt[1], Thorben Nawrath[2], Stefan Schulz[2], André Fleissner[1]. 1) Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany; 2) Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany.

Sterols are major constituents in the plasma membrane of eukaryotic cells. They modulate the physical properties of the lipid bilayer, e.g. fluidity. By interacting with certain lipids and proteins in the plasma membrane, sterols cluster into microdomains which might act as platforms for many biological functions, such as signal transduction. In the early stages of colony formation in *Neurospora crassa*, germinating spores direct their growth towards each other, establish physical contact, and fuse. Cell-to-cell signaling requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1. Here, we report that mutants affected in the biosynthesis of ergosterol, the major sterol in most fungal species, show distinct defects during germling fusion. Deletion of *erg-2*, which encodes an enzyme mediating the last step in the pathway, strongly impairs both directed growth and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting D*erg-2* germlings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, D*erg-10a* D*erg-10b* germling pairs show D*Prm1*-like deficiencies in plasma membrane merger. By relating the sterol composition and fusion competence of several *erg* mutants, we find that not the absence of ergosterol but the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes D*erg-2*-like deficiencies, an altered double bond arrangement in the sterol ring system causes D*Prm1*-like defects. During sexual development, cell fusion precedes the fertilization of fruiting bodies. Unlike the defects during germling fusion, female and male mating partners of D*erg-2* and D*erg-10a* D*erg-10b* efficiently fuse, suggesting that alterations in the sterol composition specifically impair signaling mechanisms mediating vegetative cell fusion. These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signaling complexes, and plasma membrane fusion.

Co-option of a sex pheromone receptor and MAPK signalling pathway for chemotropism of Fusarium oxysporum towards plant host compounds. David Turra, Federico Rossi, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain.

Fungal hyphae explore the surrounding environment in search of nutrient sources, mating partners or host organisms by sensing gradients of tropically active cues. Chemotropism is crucial for fungal development and virulence, but the underlying mechanisms are poorly understood. Here we followed a genetic approach to dissect chemotropism in the soilborne plant pathogen Fusarium oxysporum. A plate assay was used to measure directed growth of germ tubes towards different classes of compounds, including carbon and nitrogen sources, sex pheromones, plant secondary metabolites and tomato root exudate. F. oxysporum mutants lacking the mitogen activated protein kinase (MAPK) Fmk1 or the transcription factor Ste12, two components of the conserved Pathogenicity MAPK cascade, were impaired in chemotropism towards nutrients, but fully responsive to a-pheromone and root exudate. By contrast, Rho1 and Mpk1, two components of the cell integrity MAPK cascade, were specifically required for directed growth towards a-pheromone and root exudate. Deletion of the seven transmembrane G protein coupled receptor Ste2 abolished the chemotropic response to a-pheromone and, unexpectedly, also to tomato root exudate. Our results provide evidence for co-option of a cognate sex pheromone receptor and a conserved MAPK signalling pathway for chemotropism of F. oxysporum towards plant host compounds.

Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete *Sordaria macrospora*. Britta Herzog, Yasmine Bernhards, Berit Habing, Eva Reschka, Sabine Riedel, Stefanie Pöggeler. Institute of Microbiology and Genetics, Department of Genetics of Eurkaryotic Microorganisms, Georg-August-University Göttingen, Germany.

Using *Sordaria macrospora* as model organism we investigate the complex process of fruiting-body development and involved proteins in this filamentous ascomycete. This differentiation process is regulated by more than 100 developmental genes. Recently, we have shown that a homologue of the human STRIPAK (striatin-interacting phosphatase and kinase) complex engages a crucial role in sexual development in fungi. The *S. macrospora* striatin homologue PRO11 and its interaction partner SmMOB3 are key components of this complex (Bloemendal *et al*., 2012). PRO11 contains a conserved WD40 repeat domain and is supposed to function as scaffolding protein linking signaling and eukaryotic endocytosis (Pöggeler and Kück, 2004). SmMOB3 (phocein) is a member of the MOB family (Bernhards and Pöggeler, 2011). Beside their important role in multicellular development and hyphal fusion both proteins seem to be involved in vesicular trafficking and endocytosis.

By means of yeast two-hybrid screens and GFP-Trap analysis we identified several new interaction partners of PRO11 and SmMOB3. Similar to PRO11 and SmMOB3, a multitude of them are predicted to be involved in vesicular trafficking and are localized to the ER or to the Golgi. Here, we show the results of a detailed analysis of the new STRIPAK complex interaction partners. Initially, we isolated the cDNA of the genes and confirmed the interaction by yeast two-hybrid. For further characterization and to get knowledge about their cellular functions we created knock-out strains and analyzed their morphological phenotypes. For localization and expression studies we constructed EGFP-tagged fusion proteins and expressed them in *S. macrospora*.

Bernhards and Pöggeler, 2011; Curr Genet 57 (2): 133-49.
Bloemendal *et al*., 2012; Mol Microbiol 84 (2): 310-23.
Pöggeler and Kück, 2004; Eukaryot Cell 3 (1): 232-40.

Characterisation of contact-dependant tip re-orientation in *Candida albicans* hyphae. Darren Thomson, Silvia Wehmeier, Alex Brand. Aberdeen Fungal Group, Aberdeen University, Aberdeen, United Kingdom.

Candida albicans is a pleiomorphic fungus that lives as a commensal yeast in the human body but can become pathogenic in susceptible patient groups. Virulence is strongly linked with the production of penetrative hyphae that can adhere to and invade a wide range of substrates, including blood vessels, organ tissue, keratinised finger-nails and even soft medical plastics. Using live-cell imaging and nanofabricated surfaces, we are characterising the spatio-temporal dynamics of contact-induced hyphal tip behaviour (thigmotropism). To test whether tip re-orientation responses positively correlate with levels of hyphal adhesion, we generated substrates with increasing adhesive force. Hyphal tip re-orientation was absent in poorly-immobilised hyphae and a threshold adhesive force was required sub-apically to generate the hyphal tip pressure required for re-orientation. Interestingly, sub-threshold adhesion resulted in sub-apical hyphal bending. Localization of fluorescent protein markers for the Spitzenkörper and the Polarisome (Mlc1-YFP and Spa2-YFP, respectively) showed that *C. albicans* hyphal tips grow in an asymmetric, 'nose-down' manner on a surface. Additionally, hyphal tips can detect surface stiffness and show a distinct preference for nose-down growth on the softer of two substrates. Localisation of fluorescent cell-cycle reporter proteins over time revealed that hyphal tip contact slowed the cell-cycle, suggesting that tip-contact perturbs cell-cycle mechanics. Finally, we examined the role of cytoskeleton regulators in thigmotropism and determined the force that can be generated by the hyphal tip. Our results suggest that *C. albicans* hyphae can exert sufficient force to penetrate human epithelial tissue without the need for secreted enzyme activity. This is consistent with the observed hyphal penetration of medical-grade silicone, which has a similar Young's modulus to human cartilage.

Biochemistry and Metabolism

1. Heme regulation in *Aspergillus fumigatus*., Nicola Beckmann

2. Key Steps in the Biosynthesis of the Fungal Virulence Factor Gliotoxin., Pranatchareeya Chankhamjon

3. Identification of a gene cluster mediating the biosynthesis of the *Aspergillus fumigatus* cell wall and secreted polysaccharide, galactosaminogalactan., Fabrice N. Gravelat

4. A fasciclin-like protein in *Aspergillus fumigatus*., Thomas Hartmann

5. Characterization of fumiquinazoline biosynthesis in *Aspergillus fumigatus*., Fang Yun Lim

6. Identification of local and cross-chromosomal biosynthetic gene clusters in filamentous fungi using gene expression data. Mikael R. Andersen

7. N-glycan profiling of *Aspergillus nidulans* using solid-phase glycan extraction and mass spectrometry. Diana Anyaogu

8. Targeting of AcvA to peroxisomes increases penicillin production in *Aspergillus nidulans*. Andreas Herr

9. Characterization of the 3-methyl orsellinic acid gene cluster in *Aspergillus nidulans*. Jakob B. Nielsen

10. Induction of sclerotia and *Aspergillus* section *Nigri*. Jens Frisvad

11. Analyzing the impact of compartmentalization on organic acid production in *Aspergillus niger*. Matthias G. Steiger

12. Subcellular localization of aphidicolin biosynthesis enzymes from *Phoma betae* expressed heterologously in *Aspergillus oryzae*. K. Gomi

13. Increased production of fatty acids and triglycerides in *Aspergillus oryzae* by modifying fatty acid metabolism. Koichi Tamano

14. Improved Properties of Thermostable Cellobiohydrolase in a Treatment of Cellulosic Material. Taija Leinonen

15. The phytopathogenic fungus Botrytis pseudocinerea is resistant to the fungicide fenhexamid due to detoxification by a cytochrome P450 monooxygenase Cyp684. Danièle Debieu

16. Evolutionary rewiring of ubiquitination targets in *Candida albicans* promotes efficient carbon assimilation in host niches. Alistair J. P. Brown

17. Can-Hsp31 is important for *Candida albicans* growth and survival. S. Hasim

18. Influence of N-glycans on α-/β-(1,3)-glucanase and α-(1,4)-amylase from Paracoccidioides brasiliensis yeast cells. Fausto Bruno Dos Reis Almeida

19. Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis. Vincent Bulone

20. Investigating the function of a putative chitin synthase from *Phytophthora infestans*. Stefan Klinter

21. Deciphering cell wall structure and biosynthesis in oomycetes using carbohydrate analyses and plasma membrane proteomics. Hugo Melida

22. Identification and characterization of the chitin synthase genes in the fish pathogen *Saprolegnia parasitica*. Elzbieta Rzeszutek

23. Role of Ccr4-mediated mRNA turnover in nucleotide/deoxynucleotide homeostasis and Amphotericin B susceptibility in Cryptococcus neoformans. D. Banerjee

24. *WITHDRAWN*

25. Blue light induce Cordyceps militaris fruiting body formation and cordycepin production. Chun-Hsiang Yang

26. Insight into alkaloid diversity of the epichloae, protective symbionts of grasses. Carolyn A. Young

27. Extracellular polysaccharide degrading capabilities of various *Agaricus bisporus* strains during compost cultivation. A. Patyshakuliyeva

28. Reconstruction of the rubrofusarin biosynthetic pathway in *Saccharomyces cerevisiae*. Rasmus J. N. Frandsen

29. Expression and purification of hydrophobin fusion proteins targeted to intracellular protein bodies in *T. reesei* . Nina K. Aro

30. Metabolic adaptations in *Phytophthora infestans* and the role of a phosphagen kinase system in energy metabolism. Meenakshi Kagda

31. Platforms for secondary metabolite analysis in filamentous fungi. Uffe H. Mortensen

32. Transcriptional analysis of oxalate degradation in the white rot basidiomycete *Dichomitus squalens*. Miia R. Mäkelä

33. Creation of temperature-influenced hyphal growth mutants in a basidiomycete fungus through the use of UV mutagenesis. Stephen J. Horton

34. Functional Analysis of a Novel Diaminopimelate Decarboxylase from the Oomycete Saprolegnia parasitica. Paul Morris

35. Living on Air?: *Ustilago maydis* cells grow without being provided nitrogen in their growth media. Michael H. Perlin

36. Saprotrophic metabolism of the White-Nose Syndrome fungus *Geomyces destructans* in bat hibernacula. Hannah Reynolds

37. Cellulose acting enzymes of the white-rot fungus *Dichomitus squalens*: expression of the genes and characterization of the enzymes. Johanna Rytioja

38. Metabolomics of growth and type B trichothecenes production in *Fusarium graminearum*. N. Ponts

39. Diversity of telomeric sequences and telomerase RNA structures within Ascomycetes. Xiaodong Qi

40. Characterizing a putative three-step formaldehyde oxidation pathway in *Neurospora crassa*. Ethan Addicott

41. Nitrate assimilation in *Neurospora crassa*. Oleg Agafonov

42. Protein kinases affecting glycogen accumulation and likely regulating the glycogen synthase phosphorylation status in *Neurospora crassa*. T. Candido

43. Endogenous ergothioneine is required for wild type levels of *Neurospora crassa* conidiogenesis and conidial survival, but does not protect against uv-induced kill or mutagenesis. Lynn Epstein

44. Thiolutin inhibits protein turnover in Neurospora and yeast. Linda Lauinger

45. Characterization of a Phanerochaete chrysosporium glutathione transferase reveals a novel structural and functional class with ligandin properties for wood extractive molecules. Melanie Morel

46. Secretome analysis of *Trichoderma harzianum* cultivated in the presence of *Fusarium solani* cell wall or glucose. Marcelo HS Ramada

47. Analysis of carbon catabolite repression (CCR) during cellulase formation by *Trichoderma reesei (Hypocrea jecorina*) using two-dimensional differential gel electrophoresis (2D-DIGE). Roberto N. Silva

48. Transposon-associated evolution of a fungal NRPS. Daniel Berry

49. Velvet family control of penicillin production in *Penicillium chrysogenum*: PcVelB binding to isopenicillin N synthase suggests a novel regulatory mechanism. Sandra Bloemendal

50. Genome mining reveals the evolutionary origin and biosynthetic potential of basidiomycete polyketide synthases. Gerald Lackner

51. Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms. Hong Luo

52. Spatial assessment of oxidative and enzymatic reactions in brown rotted wood. Jon R. Menke

53. Molecular biological basis for statin resistance in naturally statin-producing organisms. Ana Rems

54. Molecular genetic characterization of secondary metabolism pathways in Asperillus species. Clay Wang

55. A branched biosynthetic pathway is involved in production of roquefortine and related compounds in Penicillium chrysogenum. Arnold Driessen

56. A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, *Leptosphaeria maculans*. Candace Elliott

57. Exploring and Manipulating Pleuromutilin Production. Patrick M. Hayes

58. Improvement of *Monascus pilosus* for the production of functional foods by overexpression of the *laeA* gene. In H. Lee

59. Molecular genetics studies on secondary metabolism in *Chaetomium globosum* reveal involvement of aureonitol and chaetoglobosins in gene regulation and sexual reproduction. Takehito Nakazawa

60. Identification of T. asperellum CAZyme genes. Lasse Bech

61. Identification of a lactose permease of Trichoderma reesei that is required for cellulase gene expression. Christa Ivanova

62. The diversity of the Mannosylerythritol lipids depends on the peroxisomal targeting of the Mannosylerythritol acyl transferases Mac1 and Mac2 in *Ustilago maydis*. Björn Sandrock

63. Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers. Carol E. Davis

64. Multi-copper oxidase genes of *Heterobasidion irregulare*. Bastian Doernte

65. The two novel class II hydrophobins of *Trichoderma* stimulate enzymatic hydrolysis of polyethylene terephthalate (PET). Irina S. Druzhinina

66. Analysis of polyketide synthase gene clusters in *Cladonia metacorallifera* genome. J.-S. Hur

67. Inhibition of benzoate 4-monooxygenase (CYP53A15) from *Cohliobolus lunatus* by cinnamic acid derivatives. Branka Korosec

68. Higher yields of cyclodepsipetides from *Scopulariopsis brevicaulis* by random mutagenesis. Linda Paun

Cell Biology and Development

69. Generation of pathogenic diploids from heterogeneous conidial populations of Aspergillus flavus. Farhana Runa

70. Inhibition of appressorium formation of *Magnaporthe oryzae* by roxithromycin and its possible molecular target. Akira Ishii

71. Identification of novel genes involved in induction of appressorium development triggered by plant-derived signals in *Colletotrichum orbiculare*. Sayo Kodama

72. Unique protein domains regulate *Aspergillus fumigatus* RasA localization and signaling during invasive growth. Jarrod R. Fortwendel

73. Light regulates growth, stress resistance and metabolism in the fungal pathogen *Aspergillus fumigatus*. Kevin K. Fuller

74. Analysis of critical domains in calcineurin A required for septal targeting and function in *Aspergillus fumigatus*. Praveen R. Juvvadi

75. Calcium imaging and measurement during growth and response to stresses in *Aspergillus fumigatus*. Alberto Muñoz

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77. The copper transporter *ctpA* in *Aspergillus fumigatus* is critical for conidial melanization and virulence in an invertebrate infection model. Srijana Upadhyay

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79. The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. Oezguer Bayram

80. Functional analysis of sterol transporter in filamentous fungus Aspergillus nidulans. Nicole Bühler

81. Mechanisms of cellular resistance to copper and arsenic in Aspergillus nidualans. Steven H. Denison

82. Functional characterization of *Aspergillus nidulans* ANID_05595.1: a possible homologue of the polarisome component Pea2. Nathan W. Gross

83. *Aspergillus nidulans* septin interactions and post-translational modifications. Yainitza Hernandez-Rodriguez

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85. The MpkB MAP kinase plays a role in autolysis and conidiation of *Aspergillus nidulans*. Kwang-Yeop Jahng

86. Beyond green mining: analysis of fungal cytochemistry using gold nanoparticles. Susan GW Kaminskyj

87. *Aspergillus nidulans* as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug resistance mutations. Susan Kaminskyj

88. *Aspergillus nidulans* cell walls lacking galactofuranose are more susceptible to glucan degrading enzymes. Susan Kaminskyj

89. The GATA-type transcription factor NsdD is a key regulator of conidiation and secondary metabolism in *Aspergillus*. Mi-Kyung Lee

90. THE *velvet* regulators in *Aspergilli*. Heesoo Park

91. Coordinated regulation of asexual development, cell death and autolysis by the C2H2 zinc finger transcription factor BrlA in *Aspergillus nidulan*. István Pócsi

92. Whole-genome sequencing identifies novel alleles of genes required for organelle distribution and motility in *Aspergillus nidulans*. Samara Reck-Peterson

93. Two methyltransferase protein complexes control fungal development and secondary metabolite production. Oezlem Sarikaya Bayram

94. Control of Multicellular Development by the Physically Interacting Deneddylases DEN1/DenA and COP9 Signalosome. Josua Schinke

95. Visualization of apical membrane domains in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM). Norio Takeshita

96. Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function. Lakshmi Preethi Yerra

97. The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in *Aspergillus*. Jae-Hyuk Yu

98. Evidence for a role of peroxisomes in microtubule organization. Ying Zhang

99. Autophagy promotes survival in aging submerged cultures of the filamentous fungus *Aspergillus niger*. Maria A. Burggraaf

100. Inactivation of *flbA* results in increased secretome complexity and reduced secretion heterogeneity in colonies of *Aspergillus niger*. Pauline Krijgsheld

101. Functional characterization of *A. niger* class III and class V chitin synthases and their role in cell wall integrity. Jean-Paul Ouedraogo

102. Exploiting transcriptomic signatures of Aspergillus niger to uncover key genes important for high protein traffic through its secretory pathway. Joohae Park

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104. Maltose permease-encoding mRNA is cleaved under induction condition of amylolytic gene expression in *Aspergillus oryzae*. Mizuki Tanaka

105. Functional characterisation of Rac GTPase in *Botrytis cinerea* reveals impact on polarity, cell cycle and pathogenicity. Anna Minz-Dub

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107. Functional analysis of genes in the mating type locus of *Botrytis cinerea*. Jan van Kan

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109. The *pescadillo* homolog, controlled by Tor, coordinates proliferation and growth and response in *Candida albicans* yeast. Tahmeena Chowdhury

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111. Characterisation of contact-dependant tip re-orientation in *Candida albicans* hyphae. Darren Thomson

112. Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase. Audrey M. V. Ah-Fong

113. *Colletotrichum orbiculare* Bub2-Bfa1 complex, a spindle position checkpoint (SPOC) component in *Saccharomyces cerevisiae*, is involved in proper progression of cell cycle. Fumi Fukada

114. Metazoan-like mitotic events in the basidiomycetous budding yeast *Cryptococcus neoformans* - a human fungal pathogen. L. Kozubowski

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116. Investigating Cell Cycle-Regulated Control of Appressorium Morphogenesis in the Rice Blast Fungus *Magnaporthe oryzae*. Wasin Sakulkoo

117. THE ROLE AND TRAFFIC OF CHITIN SYNTHASES IN *Neurospora crassa*. R. Fajardo

118. DFG5 and DCW1 cross-link Cell Wall Proteins into the Cell Wall Matrix. Stephen J. Free

119. Cell wall biology to illuminate mechanisms of pathogenicity in

***Phytophthora infestans*.** Laura Grenville-Briggs

120. Analysis of the cell wall integrity (CWI) pathway in *Ashbya gossypii*. Klaus B. Lengeler

121. Dynamics of exocytic markers and cell wall alterations in an endocytosis mutant of *Neurospora crassa*. Rosa R. Mouriño-Pérez

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125. Black holes in fungal virulence: loss of RNAi in C. gattii outbreak strains reveals a novel RNAi factor. Marianna Feretzaki

126. The Crz1/Sp1 transcription factor of Cryptococcus neoformans is activated by calcineurin and regulates cell wall integrity. Sophie Lev

127. A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrinal Signaling. Linqi Wang

128. The PacC Signal Transduction Pathway regulates Sexual Development in Neurospora crassa. Stephen J. Free

129. *Aspergillus flavus* MAP kinase AflMpkB positively regulates developmental process but not aflatoxin production. Kap-Hoon Han

130. Subcellular localization and kinase activity of GK4, a Phytophthora infestans GPCR-PIPK involved in actin cytoskeleton organisation. Chenlei Hua

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132. Functional analysis of carbonic anhydrases from the filamentous ascomycete *Sordaria macrospora*. Ronny Lehneck

133. The *Coprinopsis cinerea cag1* (*cap-growthless1*) gene, whose mutation affects cap growth in fruiting body morphogenesis, encodes the budding yeast Tup1 homolog. H. Muraguchi

134. Adaptation of the microtubule cytoskeleton to multinuclearity and chromosome number in hyphae of *Ashbya gossypii* as revealed by electron tomography. P. Philippsen

135. High resolution proteomics of spores, germlings and hyphae of the phytopathogenic fungus *Ashbya gossypii*. P. Philippsen

136. Indoor Fungal Growth and Humidity Dynamics. Frank J. J. Segers

137. Essentiality of Ku70/80 in Ustilago maydis is related to its ability to suppress DNA damage signalling at telomeres. Jose Perez-Martin

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141. Biochemical and biophysical analysis of the CarO rhodopsin of *Fusarium fujikuroi*. Jorge García-Martínez

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144. Functional analysis of Elongator complex protein 3 in *Gibberella zeae*. Y. J. Lee

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147. A systems-biology approach to build gene-regulatory network models connecting osmotic stress responses and asexual development in *Fusarium graminearum*. A. Thompkins

148. Starvation enhances heterokaryon formation between incompatible strains of *Fusarium oxysporum*. Shermineh Shahi

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150. Characterization of the endocytotic proteins Yel1-Arf3-Gts1 in *Ashbya gossypii* and the role of Gts1 in endocytosis, actin localization and filamentous growth. Therése Oskarsson

151. A Late Embryogenesis Abundant (LEA) protein in *Neosartorya fischeri* confers protection against desiccation. Martin Richard van Leeuwen

152. Coordination of polarized secretion by the exocyst complex is critical for filamentous growth and cytokinesis in *Ustilago maydis*. Michaela Wehr

153. Localization of Neurospora crassa Cell Fusion Proteins. Ci Fu

154. Identification of novel *Neurospora crassa* genes involved in hyphal fusion by tanscriptomic analysis. Wilfried Jonkers

155. *N*-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi. Sarah A. Gilmore

156. How water influences fungal growth on "real" materials. H. P. Huinink

157. Identification and characterization of two genes required in the control of a cell degeneration in the filamentous fungi *Podospora anserina*. Herve Lalucque

158. Dynein drives oscillatory nuclear movements in the phytopathogenic fungus *Ashbya gossypii* and prevents nuclear clustering. P. Philippsen

159. Quantification of the thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography. Karen Stephenson

160. Specificity determinants of GTPase recognition by RhoGEFs in *Ustilago maydis*. Britta A. M. Tillmann

161. Moisture dependencies of *P. Rubens* on a porous substrate. K. A. van Laarhoven

162. Localization of Gα proteins during germination in the filamentous fungus, Neurospora crassa. Ilva Esther Cabrera

163. Deciphering the roles of the secretory pathway key regulators YPT-1 and SEC-4 in the filamentous fungus *Neurospora crassa*. E. Sanchez

164. Functional characterization of CBM18 proteins, an expanded family of chitin binding genes in the *Batrachochytrium dendrobatidis* genome. Jason Stajich

165. The exocyst complex is necessary for secretion of effector proteins during plant infection by *Magnaporthe oryzae*. Yogesh K. Gupta

166. Functional analysis of protein ubiquitination in the rice blast fungus *Magnaporthe oryzae*. Yeonyee Oh

167. The role of autophagy in *Cryphonectria hypovirus 1* (CHV1) infection in *Cryphonectria parasitica*. M. Rossi

168. *Neurospora crassa* protein arginine methyl transferases are involved in growth and development and interact with the NDR kinase COT1. D. Feldman

169. Role of *tea1* and *tea4* homologs in cell morphogenesis in *Ustilago maydis*. Flora Banuett

170. Sex determination directs uniparental mitochondrial inheritance in *Phycomyces blakesleeanus*. Viplendra P. S. Shakya

171. Exploring the role of a highly expressed, secreted tyrosinase in Histoplasma capsulatum mycelia. Christopher F. Villalta

172. Hypobranching induced by both anti-oxidants and ROS control gene knockouts in Neurospora crassa. Michael K. Watters

173. Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites. Diego Delgado-Álvarez

174. Characterization of the *Neurospora crassa* STRIPAK complex. Anne Dettmann

175. Does the CENP-T-W-S-X tetramer link centromeres to kinetochores? Jonathan Galazka

176. Proper actin ring formation and septum constriction requires coordination of SIN and MOR pathways through the germinal centre kinase MST1. Yvonne Heilig

177. Regulatation of the BUD3-BUD4 landmark complex by the NDR kinases DBF2 and COT1 during septum formation in *Neurospora crassa*. Yvonne Heilig

178. Development of a Protein-Protein Interaction Platform in *Neurospora Crassa*. Shouqiang Ouyang

179. Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in *Neurospora crassa*. Martin Weichert

180. The role of NADPH oxidases in *Neurospora crassa* cell fusion. Nallely Cano-Dominguez

181. DYNAMICS OF THE PROTEINS BUD-2 AND BUD-5 DURING CELL POLARIZATION IN *NEUROSPORA CRASSA*. E. Castro-Longoria

182. The role of calcium and calmodulin during cell fusion and colony initiation in *Neurospora crassa*. Chia-Chen Chang

183. Deletion of cAMP phosphodiesterase *pde-2*/*acon-2* gene causes the enhanced osmotic sensitivity in *os-1* and *os-2* mutants of *N.* M. Fujimura ***crassa*.**

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186. The *N. crassa* Bem46 protein: alternative splicing and eisosomal association. Frank Kempken

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189. Localization of EGL-1 and EGL-2, two GPI anchored cell wall β (1-3) endoglucanases, at hyphal apices and septa, and in interconidial septa in *Neurospora crassa*. Leonora Martinez

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192. Dissecting the Pathway of Cellulase Secretion in Neurospora crassa. Trevor Starr

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195. Genetic analysis of the role of peroxisomes in the virulence and survival in *Fusarium graminearum*. K. Min

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200. Molecular Determinants of Sporulation in *Ashbya gossypii*. Jurgen W. Wendland

201. VELVET is regulated by ENV1 and impacts development of *Trichoderma reesei*. Hoda Bazafkan

202. Sexual reproduction and mating type function in the penicillin producing fungus *Penicillium chrysogenum*. Julia Böhm

203. Exponentiate complexity: non-mating GPCRs in the basidiomycete *Schizophyllum commune*. Daniela Freihorst

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215. Pheromone-induced G2 cell cycle arrest in Ustilago maydis requires inhibitory phosphorylation of Cdk1. Sónia M. Castanheira

216. Microtubule-dependent mRNA transport and mitochondrial protein import in Ustilago maydis. T. Langner

217. "The vacuole" of Neurospora crassa may be composed of multiple compartments with different structures and functions. Barry J. Bowman

218. Comparisons of two wild type *A* mating type loci and derived self-compatible mutants in the basidiomycete *Coprinopsis cinerea*. Ursula Kües

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220. Dynamics of the actin cytoskeleton in *Phytophthora infestans*. Harold Meijer

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221. A novel approach for functional analysis of genes in the rice blast fungus. Sook-Young Park

222. Distribution and evolution of transposable elements in the Magnaporthe oryzae/grisea clade. Joelle Amselem

223. Alternative structural annotation of Aspergillus oryzae and Aspergillus nidulans based on RNA-Seq evidence. Gustavo C. Cerqueira

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225. Genome sequencing of *Verticillium albo-atrum* pathotypes in order to understand wilt disease in hop production. J. Jakse

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227. Assembly, Annotation, and Analysis of Multiple Mycorrhizal Fungal Genomes. Alan Kuo

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235. Systematic analysis of the uncharacterized genes, which widely conserved among filamentous fungi, in *Aspergillus oryzae*. K. Iwashita

236. *Penicillium purpurogenum* degrades lignocellulose. What can we learn of this process by analyzing the genome, transcriptome and secretome of the fungus? Jaime Eyzaguirre

237. Functional genomics of lignocellulose degradation in the Basidiomycete white rot *Schizophyllum commune*. Robin A. Ohm

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239. Regulation of biofilm formation in Candida parapsilosis. Linda Holland

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241. A RNA-Seq directed functional genomics screen to identify novel cell wall genes in the hyphal tip of Neurospora crassa. Divya Sain

242. Identification of centromeres in the plant pathogen *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*). Klaas Schotanus

243. Loss of the RNAi pathway in VGII *Cryptococcus gattii* sheds light on the intact system in *Cryptococcus neoformans*. R. Blake Billmyre

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246. Identification of high temperature-regulated genes controlled by Sch9 through comparative transcriptome analysis in *Cryptococcus neoformans*. Dong-Hoon Yang

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250. Oömycetes Protein Array Project. Samantha Taylor

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254. Evolutionary genomic analysis of cytochrome P450 proteins in the subphyla Pezizomycotina. Venkatesh Moktali

255. Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis. Stephen J. Mondo

256. The Unique family of Telomere-Linked Helicases in Fungi. Olga Novikova

257. Evolution of proteins containing intein- and Hedgehog-like Vint domains in Fungi. Olga Novikova

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265. Understanding the remodeling of the wheat grain genome expression during infection, a gate to get new insights on the molecular cross-talk controlling the development of the interaction between the wheat and *Fusarium graminearum*.. Chetouhi Cherif

266. Genetic and epigenetic changes in *Fusarium graminearum* following serial subculture. Heather E. Hallen-Adams

267. Evolutionary and functional analysis of mitosis-related kinase genes in Fusarium graminearum. Huiquan Liu

268. Functional analysis of A MADS-box transcription Mcm1 in *Fusarium graminearum* . Chen fang Wang

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275. Tyrosinase an important enzyme for melanin production in the oomycete Saprolegnia parasitica. Marcia Saraiva

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279. *Anisogramma anomala*: a unique fungus with a huge genome. Guohong Cai

280. *Leptosphaeria maculans* 'brassicae': "Transposable Elements changed my life, I feel different now". Jonathan Grandaubert

281. Genomics of fungal interactions for bioenergy crops. Igor Grigoriev

282. Genomic analysis of oleaginous fungi. H. Koike

283. Genome sequencing, assembly and annotation of a marine fungal isolate of Scopulariopsis brevicaulis using three different next generation sequencing technologies. Abhishek Kumar

284. Genome sequencing, assembly and annotation of a marine fungal isolate of Pestalotiopsis using next generation sequencing technologies. Abhishek Kumar

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286. Genome sequencing, assembly and annotation of three marine fungal isolates using different next generation DNA sequencing methods for pharmaceutically important secondary metabolites. Abhishek Kumar

287. From Shiitake genomics to comparative mushroom genomics: Mushroomics. Hoi Shan Kwan

288. Comparative genomics of *Ceratocystis polonica* and *Ophiostoma bicolor*, two bark beetle-associated pathogenic fungi. Ljerka Lah

289. The largest fungal mitochondrial genome of a basidiomycete contains signs of genetic flexibility and recombination events. Taina K. Lundell

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293. The transcriptional response during cell-fusion incompatibility in Podospora anserina. Sven J. Saupe

294. Comparative Analysis of Putative Rhodopsins in Early Diverging Fungal Lineages. Steven Ahrendt

295. Molecular Tools to Silence and Confirm Genes in Phytophthora Sojae. Felipe R. Arredondo

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297. Comparative genomic analysis of world-wide *Magnaporthe oryzae* isolate collection. Jaeyoung Choi

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300. RNA-seq analyses of gene expression in the microsclerotia of *Verticillium dahliae*. Steven J. Klosterman

301. Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis. Francis M. Martin

302. Known unknown genes: evolution of eukaryotic BEM46. Abhishek Kumar

303. Sugar 'cubed' - A Comparative Systems Analysis of Plant Cell Wall Polysaccharide Recognition and Degradation Using the Model Filamentous Fungus *Neurospora crassa*. J. P. Benz

304. Building upon whole genome resequencing in Neurospora. Kevin McCluskey

305. Genome based phylogeny of early diverging fungal lineages. A. P. Gryganskyi

306. Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum. Robert Riley

307. Functional analysis of roles of expanded genes in fruiting body development in Coprinopsis cinerea. Jinhui Chang

308. Comparative analysis of fungal kinomes. Abhishek Kumar

309. VeA, VelB and FluG affect conidiation and aflatoxin production of Aspergillus flavus. L. Scharfenstein

310. The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology. S. E. Baker

311. Comparative Genomics and Transcriptomics of Insect Pathogenesis. Kathryn E. Bushley

312. Integrated transcriptional profiling and analysis for identification of *Cryptococcus neoformans* genes regulated during human cryptococcal meningitis. Y. Chen

313. Structural and functional characterization of microRNA-like RNAs in the penicillin producing fungus *Penicillium chrysogenum*. Tim Dahlmann

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555. TOR-mediated control of virulence functions in the trans-kingdom pathogen *Fusarium oxysporum*. Gesabel Y. Navarro Velasco

556. Components of the urease complex govern virulence of *Fusarium oxysporum* on plant and animal hosts. Katja Schaefer

557. Knock-out of the *Fusarium oxysporum* f.sp. *lycopersici* homologs of the DNA-methylation genes *DIM2* and *HP1* does not affect effector gene expression. Charlotte van der Does

558. Mechanistic investigation of *Trichoderma cf. harzianum* SQR-T037 mycoparasitism against *Fusarium oxysporum* f. sp. *cubense* 4, (banana wilt disease). Jian Zhang

559. Epichloënin A, a unique siderophore of Epichloae endophytes and its role in restricting fungal growth *in planta*. Linda J. Johnson

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566. *Aspergillus flavus* hypertrophy and hyphal entry by *Ralstonia solanacearum* is mediated by bacterial type three secretion system function. Joe E. Spraker

567. Vegetative hyphal fusion in epichloae endophytes. Jun-ya Shoji

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574. Characterization of the binding site and downstream targets of the *MST12* transcription factor in *Magnaporthe oryzae*. Guotian Li

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576. *Magnaporthe oryzae* AVR-Pia protein: induction of resistance reaction in *Pia* rice by the recombinant AVR-Pia and preparation of anti-AVR-Pia antibody. Y. Satoh

577. Homologous recombination causes the spontaneous deletion of *AVR-Pia* in *Magnaporthe oryzae*. T. Sone

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589. The Cpc1 (CpcA/Gcn4) regulator of the cross-pathway control of amino acid biosynthesis is required for plant infection of the vascular pathogen *Verticillium longisporum*. Susanna A. Braus-Stromeyer

590. Fungal-Specific Transcription Factor *AbPf2* Activates Pathogenicity in *Alternaria brassicicola*. Yangrae Cho

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612. The role of *Epichloe festucae* RacA interacting proteins, PakA, PakB and RhoGDI, on cell polarity in culture and synchronized growth in *Lolium perenne*. Barry Scott

613. A Host-Induced Gene Silencing Approach to Control Mycotoxin Contamination in Corn. B. H. Bluhm

614. CDiT1, a novel type of proteinaceous toxin secreted by the necrotrophic pathogen of the roots of tomato *Pyrenochaeta lycopersici*. Pierre-Henri Clergeot

615. Nonhost-specific phytotoxicity of the polyketide-derived toxin solanapyrone A produced by *Ascochyta rabiei* and *Alternaria solani*. W. Kim

616. Crosstalk of the unfolded protein response and regulatory pathways controlling pathogenic development in *Ustilago maydis*. Kai Heimel

617. Transcriptional profiling of the APSES genes in Trichophyton rubrum during growth on human nail. Elza A. S. Lang

618. Carbohydrate binding proteins of two *Leptosphaeria* pathogens of *Brassica napus*. Rohan G. T. Lowe

619. Domains for plant uptake of Ustilago maydis secreted effectors. Anupama Ghosh

620. Lipid metabolism influences virulence in *Ustilago maydis*. Scott Lambie

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622. The *U. maydis* effector Pit2 inhibits maize cysteine proteases to suppress host defense. Andre Mueller

623. The Ustilago maydis MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-peptide enrichment. Vikram Naik

624. See1 : A novel organ specific effector in the *Ustilago maydis* - maize interaction. Amey Redkar

625. Investigation of unconventionally secreted proteins in *Ustilago maydis*. Stefanie Reissmann

626. Identification of a key regulator for the developmental switch leading to sporogenesis in *Ustilago maydis*. Marie Tollot

627. Genetic characterization of virulence in the *Pyrenophora teres* f. *teres* - barley pathosystem. Timothy L. Friesen

628. Illumina-based genetic linkage map for wheat leaf rust. David L. Joly

629. The deletion of the *Histoplasma capsulatum RYP1* homolog in *Coccidioides posadasii* is avirulent. M. Alejandra Mandel

630. Cpkk2, a MEK from *Cryphonectria parasitica* is necessary for maintenance of CHV1 virus infection. M. Turina

631. Deep RNAseq of wheat leaf infection by M. graminicola identifies phase-specific in planta expressed genes and varying transcriptional contributions of fungal chromosomes. Mikaël S. Courbot

632. A genomic analysis of the infection strategies employed by *Phoma medicaginis* a necrotrophic fungal pathogen of alfalfa and the model legume *Medicago truncatula*. Angela H. Williams

633. Two G protein-coupled receptors, GprC and GprD, regulate density-dependent development in *Aspergillus flavus*. Katharyn J. Affeldt

634. Characterization of genes encoding putative secreted proteins during pathogenesis in *Magnaporthe oryzae*. Seongbeom Kim

635. The biosynthesis of oxalate is entirely dependent on oxaloacetate acetylhydrolase in *Sclerotinia sclerotiorum* . X. Liang

636. In vivo efficacy of antifungal treatment of Aspergillus terreus infections and the influence on host immune response in Galleria mellonella. Elisabeth Maurer

637. Recognition and response to non self in Podospora anserina: a model of the fungal immune system. Mathieu Paoletti

638. Increased late blight resistance in HIGS potato lines targeting a *P. infestans* gene. N. Temme

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643. Cryptococcus gattii and the origins of outbreaks. Dee Carter

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647. Genetic diversity of Australian *Pyrenophora tritici-repentis* isolates using microsatellites. Caroline Moffat

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649. Fungal community composition analysis by Internal Transcribed Spacer (ITS) sequencing using Illumina MiSeq. Robin A. Ohm

650. Estimation of genetic diversity of Ramularia collo-cygni populations using nuclear SSR markers to infer its potential to adapt to environmental changes. Marta Piotrowska

651. Alkaloid genotype profiling of tall fescue endophytes to determine influence of ancestral progenitors. J. E. Takach

652. Evolution of the pan-secretome among lineages of *Magnaporthe oryzae* attacking different host-plants. E. Fournier

653. Exploiting the high evolutionary potential of *Leptosphaeria maculans* minimises severity of blackleg disease of canola. Barbara J. Howlett

654. Experimental demonstration of Crozier's paradox in fungi. Duur K. Aanen

655. A completely unknown lifecycle in mushrooms: cyclical inbreeding and haplo-diploidy. Duur K. Aanen

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657. Co-evolution and life cycle specialization of plant cell wall degrading enzymes in a hemibiotrophic pathogen. Patrick C. Brunner

658. Recombination landscape of the plant pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*). D. Croll

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660. Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta. Pierre Gladieux

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663. Neurospora presents a model for the evolution of mating systems. Christopher Hann-Soden

664. Profiling conditionally dispensable chromosomes of the plant-pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*). Ronny Kellner

665. Take a walk on the wild side: evolutionary consequences of resistance to apple scab introgressed from a wild host. C. Lemaire

666. Genomic footprint of adaptive divergence in *Ophiostoma montium*, a fungal symbiont associated with the mountain pine beetle. J. F. Mao

667. Ecological context in symbioses: when is your enemy also your friend? Georgiana May

668. Population shifts and mating-type heterokaryosis in *Aspergillus flavus*. Rodrigo A. Olarte

669. Structural variation of trichothecene mycotoxins has resulted from multiple evolutionary processes in the fungal order *Hypocreales*. R. H. Proctor

670. Evidence for birth-and-death evolution and horizontal transfer of the fumonisin mycotoxin biosynthetic gene cluster in *Fusarium*. R. H. Proctor

671. Chemotype predominance in *Fusarium graminearum* is not directly affected by the use of the fungicides trifloxystrobin and isopyrazam. Matias Pasquali

672. Evolution of races within f.sp *lycopersici* of *Fusarium oxysporum*. BV. Chellappan

673. Detection of Mitochondrial DNA Heteroplasmy in the progeny of crossed genetically divergent isolates of Arbuscular Mycorrhizal Fungi. Maryam Nadimi

674. Evolution of mode of infection in the rice blast fungus and allied species. Ning Zhang

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677. Rapid evolution of female-biased genes: a novel example from the eukaryotic model organism *Neurospora crassa*. Hanna Johannesson

678. Fungal Community Dynamics During Biomass Degradation in the Cow Rumen Determined by ITS Sequencing. Matthias Hess

679. A Systematic and Genomic Description of *Undulatus ophiodiicola*, Formerly Referred to as Chrysosporium ophiodiicola, an Emerging Fungal Pathogen of Snakes. Mana Ohkura

680. Pair-wise linkage disequilibrium decay among linked loci suggests

meiotic recombination in natural populations of *Sclerotinia sclerotiorum*. Renuka Nilmini Attanayake

681. Population genomics of *Suillus brevipes*. Sara Branco

682. Ugly, understudied and undertreated: population genomics of the most common human fungal pathogen - the dermatophyte Trichophyton rubrum. M. Gajdeczka

683. Poppr: an R package for genetic analysis of populations with mixed reproduction. Zhian N. Kamvar

684. The heterothallic fungus *Cercospora beticola* contains fragments of both mating type genes. Melvin D. Bolton

685. Population structure from mountain to coast of two Lophodermium endophytes; a case study comparing rare and common fungal species in pine needles. Ryoko Oono

686. Fungal pathogen and endophyte genetics within the context of forest community dynamics. M.-S. Benitez

687. Discovery of Sexual Reproduction in the Black Aspergilli. Heather L. Darbyshir

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689. Understanding the cellular basis of Azole resistance in Aspergillus fumigatus. Michael J. Bromley

690. Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*. D. A. Macdonald

691. Antifungal *Pisum sativum* defensin 1 Induces a non-Apoptotic Death in *Aspergillus nidulans*. Caroline M. Fernandes

692. Is fungal secondary metabolism regulated by competing insects? Frank Kempken

693. Eisosome distribution and localization in the meiotic progeny of *Aspergillus nidulans*. V. Sophianopoulou

694. Fungal-bacterial interactions: *Bacillus subtilis* forms biofilm on *Aspergillus niger* hyphae. Isabelle Benoit

695. Co-cultivations of fungi: microscopic analysis and influence on protein production. Isabelle Benoit

696. Improving heterologous protein production in *Aspergillus vadensis* . Ourdia Bouzid

697. Production and characterization of esterases from *Chaetomium thermophilum* and their applicability in biomass conversion. Xiaoxue Tong

698. Antioxidant adaptation by Eugenol and its derivatives and their affect on the expression of virulence in candida species. Aijaz Ahmad

699. Elevation of chitin is linked with multiparallel mechanisms in response to *C. albicans* cell wall stress. F. Nogueira

700. Prezygotic and postzygotic control of uniparental mitochondrial DNA inheritance in *Cryptococcus neoformans*. Rachana Gyawali

701. SIS, a sex genome defense mechanism operating in *Cryptococcus neoformans*. Xuying Wang

702. Effects of the use of biocontrol agent (*Phlebiopsis gigantea*) on fungal communities of *Picea abies* stumps. E. Terhonen

703. Diversity of yeast and mold species in different cheeses. Nabaraj Banjara

704. Heterologous expression and characterization of soil organic matter-specific proteases secreted by the ectomycorrhizal fungus *Paxillus involutus*. Morten N. Grell

705. Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance. Markus Künzler

706. Microfluidic platforms for monitoring interactions between fungi and bacteria. Martina A. Stöckli

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709. Chemical genetics: Discovery of novel fungicides and their targets in the phytopathogen Fusarium graminearum. G. Subramaniam

710. Functional characterization of an *Aspergillus flavus* polyketide synthase gene necessary for the synthesis of a sclerotium-specific pigment. J. W. Cary

711. Functional Analysis of the *Pleurotus ostreatus* Manganese-Peroxidase Gene Family. Yitzhak Hadar

712. Temperature- and pH characteristics of endo-cellulases in Rhizophlyctis rosea. Bo Pilgaard

713. Applying unconventional secretion of the endochitinase Cts1 to export heterologous proteins in *Ustilago maydis*. K. Schipper

714. A new method for fungal genetics: flow cytometry of microencapsulated filamentous microcolonies. D. Canovas

715. DNA methylation dynamics during development in the rice blast fungus. Junhyun Jeon

716. pH-enotype array: a novel phenomics platform for filamentous fungi. Jaejin Park

717. GFP analysis of meiotic recombination in Neurospora Δ*msh-2* homozygotes. David E. A. Catcheside

718. Residual recombination in *Neurospora crassa spo11* mutant homozygotes occurs during meiosis. David E. A. Catcheside

719. Controlled synthesis of gold nanoparticles by *Neurospora crassa* extract and their SERS properties. Katrin Quester

720. Cellulase production in *Neurospora crassa*. M. Reilly

721. Identification and Functional analysis of New Neurospora crassa Nonself Recognition Loci. Jiuhai Zhao

722. Combinatorial cationic and oxidative stresses promote the killing of *Candida albicans* cells by human neutrophils. Alistair J. P. Brown

723. Phosphoproteomic analysis of the aquatic fungus *Blastocladiella emersonii* during germination. J. Crestani

724. Towards an accurate genome: high-throughput proteogenomic validation of *Stagonospora nodorum* genes via sub-cellular proteomics. Kar-Chun Tan

725. Evolutionary Imprint of Fungal PKS-NRPS Catalytic Domains. Daniela Boettger

726. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. Jonathan M. Palmer

727. Overproduction of phleichrome by synthetic inducers and cloning of polyketide synthase genes in phytopathogenic fungus *Cladosporium phlei*. D.-H. Kim

728. Symbiotic fungal endophytes that confer tolerance for plant growth in saline soil. Susan GW Kaminskyj

729. Stable cesium and radiocesium response of Schizophyllum commune. Matthias Gube

730. The completion of meiosis in *Ustilago maydis* requires an Ndt80 ortholog. B. J. Saville

731. Impact of changes in the target P450 CYP51 enzyme associated with altered triazole-sensitivity in the Wheat pathogen *Mycosphaerella graminicola*. Michael Csukai

732. Molecular Evolutionary Analysis and Synteny of Fungal GAL Genes. Julien S. Gradnigo

733. Meiotic Drive: A Single Gene Conferring Killing and Resistance in Fungal Spore Killer. Pierre Grognet

734. Alkaliphilic fungi from soda lakes and soda soils. Alexey A. Grum-Grzhimaylo

735. A new method for gene mining and enzyme discovery. Y. Huang

736. Occurrence of dsRNA mycovirus (LeV-FMRI 2427) in edible mushroom *Lentinula edodes* and its meiotic stability. J.-M. Kim

737. Analysis of fungal communities associated with grapevine wood diseases, based on fungal ITS pyrosequencing. Nicolas Lapalu

738. The Antidepressant Sertraline Provides a Promising Therapeutic Option for Neurotropic Cryptococcal Infections. Xiaorong Lin

739. Analysis of gene expression of proteases of *Trichoderma* spp during confrontation with plant pathogens in vivo. Valdirene Monteiro

740. Analysis of differential protein profile during antagonism of *Trichoderma harzianum* and *Sclerotinia sclerotiorum*. Valdirene Monteiro

741. Effector proteins in fungal defense against fungivorous nematodes: Diversity and regulation of expression. D. F. Plaza

Biochemistry and Metabolism

1. Heme regulation in *Aspergillus fumigatus*. Nicola Beckmann[1], Ernst R. Werner[2], Hubertus Haas[1]. 1) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Austria; 2) Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Austria.

Sufficient iron supply is indispensable for survival of almost all organisms. However, an excess of iron is potentially toxic. In the opportunistic human-pathogenic fungus *Aspergillus fumigatus* the ability to adapt to iron limitation represents a crucial virulence factor. Iron regulation is tightly interconnected with heme metabolism, as iron-containing heme is an essential cofactor of a variety of cellular processes, e.g. respiration, sterol biosynthesis, oxidative stress detoxification and also reductive iron assimilation. Most knowledge on fungal heme regulation derives from studies in *Saccharomyces cerevisiae*. *A. fumigatus*, as well as most other fungal species, lack homologs of key heme regulators found in *S. cerevisiae*. The goal of this study is to elucidate heme-dependent regulation in *A. fumigatus* (wt). As a first step, we generated a mutant strain (*DhemA*) lacking the gene encoding aminolevulinic acid synthase (HemA), which catalyzes the committed step in heme biosynthesis. This mutation offers the possibility to control the cellular heme content by supplementation with aminolevulinic acid (ALA). Growth of *DhemA* was blocked at ALA concentrations below 20 mM, but fully restored by addition of 200 mM on solid as well as in liquid media. Supplementation with protoporphyrin IX (PpIX), the iron free heme precursor, and hemin (chloroprotoporphyrin IX iron(III)) supported growth of *DhemA*, proving that *A. fumigatus* is able to utilize exogenous porphyrins. Nevertheless, *A. fumigatus* in contrast to several other fungal species is not able to utilize hemin as iron source. Under iron starvation, ALA supplementation led to a tremendous accumulation of PpIX in both *DhemA* and wt, which indicates that HemA represents the major rate limiting step in heme biosynthesis when iron is scarce. In *DhemA*, ALA restriction transcriptionally increased the heme-biosynthetic coproporphyrinogen(III)oxidase and the putative heme receptor CFEM3. Additionally, ALA limitation decreased the resistance of *DhemA* to oxidative stress and the triazole antifungal drug posaconazole, which underlines the crucial role of heme in detoxification and sterol biosynthesis. This work was supported by the Austrian Science Foundation grant FWF P21643-B11 to HH.

2. Key Steps in the Biosynthesis of the Fungal Virulence Factor Gliotoxin. Pranatchareeya Chankhamjon[1], Daniel H. Scharf[2], Kirstin Scherlach[1], Nicole Remme[1], Andreas Habel[1], Thorsten Heinek[2], Martin Roth[3], Axel A. Brakhage[2], Christian Hertweck[1]. 1) Biomolecular Chemistry, HKI, Jena, Jena, Germany; 2) Molecular and Applied Microbiology,HKI, Jena, Jena, Germany; 3) Bio Pilot Plant,HKI, Jena, Jena, Germany.

The prototype of epipolythiodioxopiperazine (ETP) family, gliotoxin, is an infamous virulence factor of the human pathogen Aspergillus fumigatus, notably the leading cause of invasive aspergillosis in the immunocompromised patients. Its toxicity has been attributed to the unusual intramolecular disulfide bridge, which is the functional motif of all ETPs. A number of studies showed that the diketopiperazine core of gliotoxin is assembled by a non-ribosomal peptide synthetase. However, downstream pathway steps have remained elusive, mainly because of the scarcity and instability of pathway in the mediates produced. Here we present the critical role of a specialized glutathione S-transferase (GST), GliG, in the enzymatic sulfurisation and the key step of epidithiol formation by an unprecedented twin carbon-sulfur lyase, GliI. Our studies not only unveil the understanding of key steps in the biosynthesis pathway of an important virulence factor, but also outline a new function of microbial GSTs and gain insights into the formation of organosulfur compounds.

3. Identification of a gene cluster mediating the biosynthesis of the *Aspergillus fumigatus* cell wall and secreted polysaccharide, galactosaminogalactan. Fabrice N. Gravelat[1], Mark J. Lee[1], Alexander Geller[1], Dan Chen[2], Anne Beauvais[3], Hong Liu[4], William C. Nierman[2], Jean-Paul Latge[3], Thierry Fontaine[3], Scott G. Filler[4], Donald C. Sheppard[1]. 1) Microbiology & Immunology Department, McGill University, Montréal, Qc, Canada; 2) J. Craig Venter Institute, Rockville, Maryland, USA; 3) Aspergillus Unit, Institut Pasteur, Paris, France; 4) Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, USA.

Aspergillus fumigatus is the most common cause of invasive mold disease in humans. Although adherence of fungal hyphae to host constituents is a critical early step in the pathogenesis of invasive aspergillosis, the molecular mechanisms underlying this process have not been elucidated. Using a forward genetic approach, we identified a glucose epimerase, Uge3, which is required for adherence of hyphae to a wide variety of substrates. Biochemical analyses confirmed that Uge3 is required for the synthesis of the secreted glycan galactosaminogalactan (GAG), which in turn functions as the dominant adhesin of *A. fumigatus* hyphae and is required for virulence. However, the biochemical and regulatory pathways governing GAG synthesis remain unknown. Using comparative transcriptome analysis, we found that *uge3* is found within a cluster of 5 co-regulated genes on chromosome 3. Interestingly, 3 of the 5 proteins (Uge3, Gtb3 and Ega3) encoded by these genes are predicted to contain conserved domains involved in polysaccharide metabolism. The 2 other proteins (Sph3 and Esr3) have no homologs in other organisms. We hypothesized that this cluster of genes may be required for GAG biosynthesis. To test this hypothesis, we constructed deletion mutants of two of the cluster genes: *sph3*, encoding a cell surface spherulin 4-like protein; and *esr3*, encoding an extracellular serin-rich protein. Phenotypic analysis of both the D*esr3* and D*sph3* mutant strains confirmed that deletion of these genes resulted in both impaired GAG production and impaired adherence, similar to the phenotype of the D*uge3* mutant strain. Gene deletion for the 2 remaining genes is ongoing. Collectively, these data suggest that the 5 gene cluster identified on chromosome three is likely a carbohydrate biosynthetic cluster required for the synthesis of GAG. Importantly, this is the first description of a gene cluster for the biosynthesis of a cell wall polysaccharide in *A. fumigatus*, and suggests the possibility that other similar gene clusters may govern the synthesis of glycans in this fungus. The discovery of this cluster, and the subsequent characterization of the role of each of the component elements, may provide insight into the synthesis and function of GAG.

4. A fasciclin-like protein in *Aspergillus fumigatus*. Thomas Hartmann[1], Lei Sun[2], Cheng Jin[1]. 1) Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

The saprophytic filamentous fungus *Aspergillus fumigatus* has been gaining importance as an opportunistic human pathogen over the past decades, as advances in modern medicine have created a growing group of patients susceptible to potentially deadly invasive aspergillosis. The role of fungal adhesion during infection progression is still poorly understood and this work aims to focus on this neglected aspect of the infection process. Fasciclin-like proteins can be found in a wide variety of organisms, where they often perform functions related to surface adhesion. Here we describe a fasciclin-like protein in *A. fumigatus*. The Fas protein was first discovered as differentially expressed in an O-mannosyltransferase *Dpmt1* deletion strain, which indicates that it may be substantially glycosylated. We generated *Dfas* deletion strains in two different *A. fumigatus* strain backgrounds. The *Dfas* deletion strains grew normally on plates and in solution and neither bright field microscopy, nor TEM revealed phenotypical differences to the wildtype strains. Interestingly, the *Dfas* deletion strains showed reduced adhesion to hydrophobic plastic surfaces, but adhered normally to glass slides. Whether these altered adhesive properties have an effect on the strains' virulence in mammalian hosts such as mice remains to be investigated.

5. Characterization of fumiquinazoline biosynthesis in *Aspergillus fumigatus*. Fang Yun Lim[1], Brian Ames[2], Christopher Walsh[2], Nancy Keller[1]. 1) Medical microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 2) Biological chemistry and molecular pharmacology, Harvard Medical School, Boston, MA.

The fumiquinazolines (FQs) comprise a related, sequentially generated family of bioactive peptidyl alkaloids that are signature metabolites of *Aspergillus fumigatus*. The FQ framework is built by nonribosomal peptide synthetase (NRPS) machinery with anthranilate as a key non-proteinogenic amino acid building block. Despite being prevalent across the species, its gene cluster has not been characterized. Prior bioinformatic analysis coupled with heterologous expression of the putative *A. fumigatus* proteins termed here FmqA -FmqD led to the identification of a four-enzymatic process that builds increasingly complex FQ scaffolds. Briefly, FmqA, a trimodular NRPS condenses alanine, tryptophan, and anthranilic acid to form fumiquinazoline F (FQF). The tandem action of a flavoprotein (FmqB) and a monomodular NRPS (FmqC) converts FQF to fumiquinazoline A (FQA). Finally, FmqD, a FAD-dependent oxidoreductase converts FQA to the heptacyclic fumiquinazoline C (FQC). Interestingly, FmqD contains an N-terminus signal peptide predicted for extracellular transport. This study is aimed at providing *in vivo* validation to the FQ biosynthetic framework and characterizing how cellular localization of FmqD affects production of FQC in *A. fumigatus*. We found that the conidial metabolite, FQC, is the predominant FQ moiety in two wild type isolates and is selectively accumulated in the conidia. Targeted single gene deletions of FmqA through FmqD coupled with metabolomic profiling of the single biosynthetic gene mutants supported previous biochemical prediction of FQ biosynthesis. Fluorescent microscopy of mutants bearing a C-terminal FmqD-GFP fusion showed that FmqD is localized to the cell wall of the fungus and this localization is abolished when the signal peptide is removed. Future studies will elucidate if cell wall localization of FmqD is crucial for FQC production.

6. Identification of local and cross-chromosomal biosynthetic gene clusters in filamentous fungi using gene expression data. Mikael R. Andersen[1], Jakob B. Nielsen[1], Andreas Klitgaard[1], Lene M. Petersen[1], Tilde J. Hansen[1], Lene H. Blicher[1], Charlotte H. Gottfredsen[2], Thomas O. Larsen[1], Kristian F. Nielsen[1], Uffe H. Mortensen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark.

Biosynthetic pathways of secondary metabolites from fungi are currently subject to an intense effort to elucidate the genetic basis for these compounds due to their large potential within pharmaceutics and synthetic biochemistry. The preferred method is methodological gene deletions to identify supporting enzymes for key synthases one cluster at a time.

In earlier work we presented a method for using a gene expression compendium to accurately predict co-regulated gene clusters in general, and in particular the members of gene clusters for secondary metabolism. A benchmarking of the method in *Aspergillus nidulans* by comparison to previous gene deletion studies showed the method to be accurate in 13 out of 16 known clusters and nearly accurate for the remaining three.

In this work, we have expanded the algorithm to identify cross-chemistry between physically separate gene clusters (super clusters), and validate this both with legacy data and experimentally by prediction and verification of a new supercluster consisting of the non-ribosomal peptide synthetase (NRPS) AN1242 (on chr VIII) and the prenyltransferase AN11080 (on chromosome V) as well as identication of the shared product compound nidulanin A.

We also propose further implications of the gene clustering, as our analysis shows that approximately 10 % of the genes seem to be non-randomly ($p<0.05$) co-regulated with more than two neighboring genes.

We have employed *A. nidulans* for our method development and validation due to the wealth of available biochemical data, but the method can be applied to any fungus with a sequenced and assembled genome, thus supporting further secondary metabolite pathway elucidation in the fungal kingdom. We furthermore present the preliminary analysis of the application of the method to *A. niger*.

7. N-glycan profiling of *Aspergillus nidulans* using solid-phase glycan extraction and mass spectrometry. Diana Anyaogu[1], Shuang Yang[2], Jakob B. Nielsen[1], Hui Zhang[2], Michael Betenbaugh[3], Uffe Hasbro Mortensen[1]. 1) Department of Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark; 2) Department of Pathology, Johns Hopkins University, Baltimore, Maryland 21287, United States; 3) Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland 21218, United States.

Filamentous fungi from the *Aspergillus* species are widely used as cell factories for the production of chemicals and enzymes, especially *Aspergillus niger* and *Aspergillus oryzae* are used as protein producers. Fungi have a high secretion capacity in comparison to other eukaryotic expression systems as algae, yeast and insect cells. The majority of the secreted proteins are glycosylated, thus glycosylation plays an important role in the secretory pathway. Glycosylation is also important in the production of therapeutic proteins as it is involved in protein stability, ligand binding, immunogenicity and serum half-life. Furthermore the efficacy of many therapeutic proteins depends on correct glycosylation. Thus, understanding the glycosylation will enable the directed glycoengineering in *Aspergilli* to improve protein production. In the present study the Solid-Phase Glycan Extraction (SPGE) method was used to isolate and purify N-glycans from the secretome and whole cell lysates from *Aspergillus nidulans*. The mass of the glycans was determined using a MALDI-TOF MS. In addition, *A. nidulans* strains with mutations in the glycosylation pathway were analyzed and compared to the reference strain. This study shows that some of the mutations had an effect on the N-glycan profile, which shifted the profile towards glycans with a lower mass. The method presented here is thus very efficient for extracting N-glycans and for quantifying the relative abundance of different N-glycans in the secretome and whole cell lysate .

8. Targeting of AcvA to peroxisomes increases penicillin production in *Aspergillus nidulans*. Andreas Herr, Reinhard Fischer. Karlsruhe Institute of Technology, Inst. for Applied Biosciences, Dept. of Microbiology, Karlsruhe, BW, Germany.

Aspergillus nidulans produces a great variety of secondary metabolites such as mycotoxins and antibiotics. The biosynthesis requires often a large number of enzymes, and the corresponding genes are located in clusters. Each cluster normally contains a specific transcription factor, whose expression induces the coordinated expression of all other genes located in the cluster. In addition to the coordinated expression, the sequential biosynthesis of a given secondary metabolite depends on the concerted action of all enzymes. Each enzyme produces the substrate for the next enzyme in the pathway and thus the spatial distribution of the enzymes and the diffusion of the low-molecular weight intermediates could be rate-limiting factors. In order to test the hypothesis whether the production of a certain metabolite could be increased by bringing some enzymes closer together, we studied penicillin biosynthesis in *Aspergillus nidulans*. The pathway comprises only three enzymes encoded by *acvA*, *ipnA* and the peroxisomal *aatA*. In order to test whether peroxisomes could serve as production organelles, we tried to target all three enzymes to these organelles. Targeting IpnA to peroxisomes caused a complete inhibition of penicillin formation. However, in the case of AcvA peroxisomal targeting, a 4-fold increase of the penicillin yield was obtained. Currently, we are trying to further increase the penicillin yield by elevating peroxisome numbers through overexpression of pexK (1). Recently, it was shown that peroxisomes may serve novel metabolic functions in fungi, since several glycolytic enzymes appear to be partially localized in peroxisomes (2). These results suggest that peroxisomes may also be used as production containers for other metabolites. (1)Kiel JA, van der Klei IJ, van den Berg MA, Bovenberg RA & Veenhuis M (2005). Fungal Genet Biol 42: 154-164. (2)Freitag J, Ast J & Bölker M (2012). Nature 485: 522-525.

9. Characterization of the 3-methyl orsellinic acid gene cluster in *Aspergillus nidulans*. Jakob B. Nielsen[1], Marie L. Klejnstrup[1], Paiman K. Jamal[1], Dorte K. Holm[1], Michael L. Nielsen[1], Anna M. Kabat[2], Charlotte H. Gotfredsen[3], Thomas O. Larsen[1], Uffe H. Mortensen[1]. 1) Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark; 2) Center for Systems Microbiology, Department of Systems Biology, Technical University of Denmark; 3) Department of Chemistry, Technical University of Denmark.

With the aim of mapping the polyketome of *Aspergillus nidulans* we have made a library of strains, which individually overexpress PKS genes from an ectopic locus. A screen of this collection on different media demonstrated that overexpression of AN6448 (*pkbA*) leads to increased production of 3-methyl orsellinic acid. An inspection of the DNA sequence surrounding this gene uncovered a putative gene cluster including a gene, AN6446 (*pkbR*), with homology to transcription factors. Based on this observation, we decided to overexpress *pkbR*. A qRT-PCR analysis of this strain was used to delineate the borders of the gene cluster as well as to stimulate formation of cichorine, cichorinic acid, nidulol and a novel cichonidulol dimer, just to name a few of the products that we have linked to this gene cluster. Subsequent deletion of all genes in the cluster has allowed us to propose a comprehensive model for the biosynthetic pathway of this cluster.

10. Induction of sclerotia and *Aspergillus* section *Nigri*. Jens Frisvad, Lene Petersen, Ellen Lyhne, Thomas Larsen. CMB, Dept Systems Biol, Kgs. Lyngby, Denmark.

The purpose of this study was to induce sclerotium production in Aspergillus niger and other black Aspergilli. Some species in Aspergillus section Nigri are known for their production of sclerotia, especially A. carbonarius, A. tubingensis (few isolates), A. sclerotioniger, A. sclerotiicarbonarius, A. costaricaensis, A. piperis, A. japonicus, and A. aculeatus. A. heteromorphus was reported in 1955 to produce sclerotia, but this could not be confirmed in later studies. There are also un-confirmed data on sclerotium production in Aspergillus niger, but often isolates reported to produce sclerotia were not A. niger anyway. Induction of sclerotium production in Aspergillus niger is important, since this may help in inducing the perfect state in this important industrial fungus. By screening several media, we were able to develop some media and use some growth conditions that induced sclerotium production in Aspergillus niger and other species hitherto not reported to produce sclerotia. Earlier French beans were suggested as inducers of sclerotium production, but we could not repeat this with any isolate of A. niger. However by using media such as white rice and brown rice or adding different fruits to CYA (Czapek yeast autolysate agar) and incubate at 25 C we were able to induce sclerotium production in certain strains of A. niger. Old strains used for citric acid production, or full genome sequenced strains, were not induced to produce sclerotia, but several fresh strains from different foods did produce abundant sclerotia on the different media, at 25 C, but not 37 C. One older classical citric acid producer from NRRL produced many sclerotia, however. Sclerotium producing isolates also contained aflavinines, confirmed by HPLC-DAD-MS-MS, secondary metabolites only produced in the sclerotia, and detected in A. niger for the first time. Other species, such as A. ibericus, A. neoniger, A. heteromorphus, A. fijiensis, A. luchuensis (formerly A. acidus), A. aculeatinus and A. saccharolyticus could also produce sclerotia on fruit media. The sclerotia contained many sclerotium-specific secondary metabolites.

11. Analyzing the impact of compartmentalization on organic acid production in *Aspergillus niger*. Matthias G. Steiger[1,2*], Marzena L. Blumhoff[1,2,3], Diethard Mattanovich[1,2], Michael Sauer[1,2]. 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria; 2) University of Natural Resources and Life Sciences, Department of Biotechnology, Muthgasse 18, 1190 Vienna, Austria; 3) University of Applied Sciences FH-Campus Vienna, School of Bioengineering, Muthgasse 86, 1190 Vienna, Austria.

Aspergillus niger is a well-established host organism for the production of carboxylic acids. Acids like citric, gluconic and oxalic acids can already be produced by *A. niger* and high titers are obtained. The formation of carboxylic acids involves the shuttling of intermediate metabolites between different intracellular compartments and utilizes different enzymatic capabilities of the respective compartment. The knowledge about the involved shuttling mechanisms and the localization of the necessary enzymes is still fragmentary. Using fluorescence microscopy, it is possible to characterize the intracellular localization of GFP tagged proteins and hence mitochondrial leader sequences can be functionally tested. In order to analyze the influence of the compartmentalization on the organic acid production, we have chosen itaconic acid as a target substance. Itaconic acid, which was selected by the US Department of Energy as one of the 12 building block chemicals for the industrial biotechnology, is currently produced by *A. terreus*. Heterologous expression of the *A. terreus cadA* gene also enables the formation of itaconic acid in *A. niger* although only low titers are obtained. We set out to characterize the influence of the compartmentalization on the productivity and re-engineered the enzymatic cascade by flipping the enzymatic activities of the cis-aconitic acid decarboxylase and aconitase between the mitochondrion and the cytosol. We will present new leader sequences for mitochondrial targeting in *A. niger* alongside with results about the positive impact of the enzymatic re-localization on the itaconic acid production.

12. Subcellular localization of aphidicolin biosynthesis enzymes from *Phoma betae* expressed heterologously in *Aspergillus oryzae*. A. Ban[1], M. Tanaka[1], R. Fujii[2], A. Minami[2], H. Oikawa[2], T. Shintani[1], K. Gomi[1]. 1) Graduate Sch Agriculture Sci, Tohoku Univ, Sendai, Japan; 2) Graduate Sch Sci, Hokkaido Univ, Sapporo, Japan.

In recent years, a lot of biosynthesis gene clusters involving in secondary metabolite biosynthesis from filamentous fungi have been revealed, and thus the attempts to produce these valuable metabolites at high yield have been actively made. To this end, *Aspergillus oryzae* is an attractive host for heterologous secondary metabolites production because of its less productivity for own secondary metabolites, which leads to the production for the metabolite of interest at a highly pure grade. Actually, the number of reports has been increasing recently, in which biosynthetic genes involved in fungal secondary metabolite biosynthesis were heterologously overexpressed in *A. oryzae*. On the other hand, it would be necessary to consider the cellular compartments where the target secondary metabolite is synthesized in filamentous fungi to produce it efficiently in the heterologous host, *A. oryzae*. However, currently there is very little knowledge about the spatial distribution of the biosynthesis enzymes of the secondary metabolite in fungi. Therefore, in this study, we examined the subcellular localization of the enzyme proteins encoded by a gene cluster involved in aphidicolin biosynthesis from *Phoma betae*, which were expressed as GFP-fusion proteins in *A. oryzae*. The gene cluster of aphidicolin in *P. betae* contains 4 genes encoding biosynthesis enzymes (geranylgeranyl diphosphate synthase [GGS], aphidicolan-16b-ol synthase [ACS], cytochrome P450 monooxygense 1 [P450-1], and P450-2), a gene for transporter (TP), and a gene for transcription factor. We constructed 4 biosynthesis enzymes and the transporter that each was fused to GFP under the a-amylase gene promoter, and introduced into *A. oryzae*. Similarly, the organelle marker proteins fused to RFP were also constructed and expressed simultaneously with GFP-fusion proteins to identify the organelle where the biosynthesis enzyme was localized. Fluorescent microscopy revealed that GGS and ACS were distributed in the cytoplasm and P450-1 was located in endoplasmic reticulum (ER). Interestingly, all of GFP-fused P450-2 was not observed in ER when only P450-2 was expressed, but mostly localized in ER when coexpressed with P450-1. In addition, TP fused to GFP was localized mainly on the plasma membrane and also rarely observed on other organelles such as vacuole.

13. Increased production of fatty acids and triglycerides in *Aspergillus oryzae* by modifying fatty acid metabolism. Koichi Tamano[1], Kenneth Bruno[2], Tomoko Ishii[1], Sue Karagiosis[2], David Culley[2], Shuang Deng[2], James Collet[2], Myco Umemura[1], Hideaki Koike[1], Scott Baker[2], Masayuki Machida[1]. 1) National Institute of Advanced Industrial Science and Technology (AIST); 2) Pacific Northwest National Laboratory (PNNL).

Biofuels are attractive substitutes for petroleum based fuels. Biofuels are considered they do not contribute to global warming in the sense they are carbon-neutral and do not increase carbons on the globe. Hydrocarbons that are synthesized by microorganisms have potential of being used as biofuels or the source compounds. In the hydrocarbon compounds synthesized by *A. oryzae*, fatty acids and triglycerides are the source compounds of biodiesel that is fatty acid methyl ester. We have increased the production by modifying fatty acid metabolism with genetic engineering in *A. oryzae*. Firstly, enhanced-expression strategy was used for the increase. For four enzyme genes related to the synthesis of palmitic acid [C16:0-fatty acid], the individual enhanced-expression mutants were made. And the fatty acids and triglycerides in cytosol were assayed by enzyme assay kits, respectively. As a result, both fatty acids and triglycerides were most synthesized in the enhanced-expression mutant of fatty acid synthase gene at 2.1-fold and 2.2-fold more than the wild-type strain, respectively. Secondly, gene disruption strategy was used for the increase. Disruptants of several enzyme genes related to long-chain fatty acid synthesis were made individually. And one of them showed drastic increase in fatty acid synthesis. In the future, further increase in the synthesis is expected by utilizing genetic engineering in *A. oryzae*.

14. Improved Properties of Thermostable Cellobiohydrolase in a Treatment of Cellulosic Material. Taija Leinonen[1], Susanna Mäkinen[1], Kari Juntunen[1], Merja Niemi[2], Juha Rouvinen[2], Jari Vehmaanperä[1], Terhi Puranen[1]. 1) Roal Oy, Rajamäki, Finland; 2) University of Eastern Finland, Department of Chemistry, Joensuu, Finland.

Production of biofuels i.e. bioethanol from lignocellulosic material is a promising alternative technology for using biomass as a renewable and clean source of energy instead of consuming limited natural resources e.g. fossil fuels, and releasing increasing amounts of CO_2. Enzymatic hydrolysis is considered to be the most promising technology for converting cellulosic biomass into fermentable sugars. Enzymatic total hydrolysis of (ligno)cellulosic substrates requires at least cellobiohydrolases, endoglucanases and beta-glucosidases. Previously cloned thermostable glycoside hydrolase family 7 cellobiohydrolase (CBHI) from *Acremonium thermophilum* was expressed in *Trichoderma reesei*. The purified *A. thermophilum* CBHI was crystallized, and the structure of the catalytic domain of the protein was determined at a resolution of 1.8 Å revealing the overall structure of the catalytic core of the enzyme to be similar with the previously determined structures of glycoside hydrolase family 7 cellobiohydrolases. In the biomass hydrolysis experiments the *A. thermophilum* CBHI, as part of the enzyme mixture, was shown to have enhancing effect on hydrolysis yield as compared to the *Trichoderma reesei* enzyme. To achieve even further improvements in thermal stability and hydrolysis performance, several single and combined amino acid mutations were designed based on the resolved 3D-structure. The data obtained from the mutants demonstrates that thermal stability and hydrolysis performance of the *A. thermophilum* CBHI protein can be increased by introducing single mutations as well as mutation combinations to the molecule.

15. The phytopathogenic fungus Botrytis pseudocinerea is resistant to the fungicide fenhexamid due to detoxification by a cytochrome P450 monooxygenase Cyp684. Saad Azeddine, Alexis Billard, Jocelyne Bach, Catherine Lanen, Anne-Sophie Walker, Sabine Fillinger, Danièle Debieu. INRA UR1290 BIOGER CCP, avenue Lucien Brétignières F78850 Thiverval-Grignon, France.

The Botrytis species complex responsible for the grey mould disease found on grapevines is composed of two species: Botrytis cinerea, to a large extent (roughly 90%), and Botrytis pseudocinera. Despite their genetic polymorphism, these species cannot be morphologically distinguished. However, they do differ in their response to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While B. cinerea is sensitive to this hydroxyanilide fungicide, B. pseudocinerea is naturally resistant. Because a strong synergism was found on B. pseudocinerea between fenhexamid and sterol 14a-demethylation inhibitors (DMIs) known to inhibit Cyp51, a cytochrome P450 monooxygenase, it was hypothetized that the detoxification of fenhexamid by a cytochrome P450 monooxygenase similar to Cyp51 is involved in the resistance B. pseudocinerea displays. To test this, we sought the gene overexpressed in the presence of fenhexamid with the highest similarity to cyp51. Taking into account the Cyp P450 classification based on homology and phylogenetic criteria, this gene, whose function remains unknown, belongs to the Cyp684 family. It was then deleted in a B. pseudocinerea strain. Cyp684 knock out mutants exhibit a loss of fenhexamid resistance and synergism between DMIs and fenhexamid, showing that the Cyp684, cytochrome P450 protein is responsible for B. pseudocinerea's natural resistance to fenhexamid and is involved in fenhexamid detoxification. Although cyp684 is also present in B. cinerea, which is sensitive to fenhexamid, a polymorphism was observed between the two species: in B. pseudocinerea the cyp684 promoter shows a deletion of 25 bp. We are currently establishing the cyp684 expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. Metabolization studies are also being conducted to identify metabolites that would help in understanding the enzymatic functions of Cyp684 and to determine to what extent Botrytis sp. is sensitive to these metabolites.

16. Evolutionary rewiring of ubiquitination targets in *Candida albicans* promotes efficient carbon assimilation in host niches. Alistair J P Brown, Doblin Sandai, Zhikang Yin, Laura Selway, David Stead, Janet Walker, Michelle D Leach, Iryna Bohovych, Iuliana V Ene, Stavroula Kastora, Susan Budge, Carol A Munro, Frank C Odds, Neil A R Gow. School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

Pathogens must assimilate carbon to grow and infect their host. Interesting questions remain about the regulation of carbon assimilation in *Candida albicans* despite the wealth of knowledge about this major fungal pathogen of humans. *C. albicans* is classified as a Crabtree-negative yeast because it continues to respire in the presence glucose [*J Med Vet Mycol* 26, 195]. How then can *C. albicans* be exquisitely sensitive to sugars, down-regulating transcripts involved in the utilization of alternative carbon sources following exposure to 0.01% glucose [*Molec Biol Cell* 20, 4845]? We have now shown that there is a significant dislocation between the transcriptome and proteome in *C. albicans*: glucose triggers the decay of key transcripts but the enzymes they encode are retained. This allows the simultaneous assimilation of alternative carbon sources such as fatty acids, carboxylic acids and sugars by *C. albicans*. This contrasts with the situation in *Saccharomyces cerevisiae* where simultaneous carbon assimilation is prevented by catabolite inactivation [*Arch Micro* 134, 187; *Arch Micro* 147, 231]. We show that *C. albicans* has retained the molecular apparatus that mediates ubiquitin-mediated, glucose-accelerated protein degradation. For example, *S. cerevisiae* isocitrate lyase (ScIcl1) is degraded rapidly when expressed in *C. albicans*. However, *C. albicans* isocitrate lyase (CaIcl1) lacks critical ubiquitination sites that mediate this catabolite inactivation. Furthermore, other *C. albicans* enzymes involved in gluconeogenesis and the glyoxylate cycle appear to lack such sites, whereas glycolytic enzymes are ubiquitinated (e.g. Fba1, Pgk1, Eno1). Therefore there has been significant rewiring of ubiquitination targets in *C. albicans* compared to *S. cerevisiae*. This metabolic flexibility probably enhances efficient colonisation of host niches that contain complex mixtures of nutrients.

17. Can-Hsp31 is important for *Candida albicans* growth and survival. S. Hasim, N. Ahmad hussin, K. Nickerson. Biological Science, Univesity of Nebraska Lincoln, Lincoln, NE.

Candida albicans is an opportunistic pathogen that is able to grow as budding yeast, pseudohyphae, and hyphae. A key feature of *C. albicans* is its ability to grow in diverse microenvironments and develop complex and highly efficient responses in order to survive within the host environment. The *C. albicans* Hsp31 (ORF19.251) gene encodes a protein that belongs to the DJ1/PfpI family with close homology to other fungal Hsp31-like proteins. Despite intensive study, the function of these fungal Hsp31 proteins is unknown. The crystal structure of Can-Hsp31 was solved to 1.6 Å resolution. Its structure is similar to those of the *E.coli* and *S. cerevisiae* Hsp31 proteins except that Can-Hsp31 is a monomer in the crystal while all other known homologues are dimers. In this report, we show that the *C. albicans*' Hsp31 is important for growth and survival under various stress conditions.

18. Influence of N-glycans on a-/b-(1,3)-glucanase and a-(1,4)-amylase from Paracoccidioides brasiliensis yeast cells. Fausto Bruno Dos Reis Almeida[1], Valdirene Neves Monteiro[2], Roberto Nascimento Silva[3], Maria Cristina Roque-Barreira[1]. 1) Cellular and Molecular Biology, University of Sao Paulo, Ribeirao Preto, Brazil; 2) University of Goias, Anapolis, Brazil; 3) Biochemistry and Immunology, University of Sao Paulo, Ribeirao Preto, Brazil.

Paracoccidioides brasiliensis (Pb) is a temperature-dependent dimorphic fungus and the causative agent of paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America. The cell wall (CW) of Pb is a network of glycoproteins and polysaccharides, such as chitin, glucan and N-glycosylated proteins, that may perform several functions. N-glycans are involved in glycoprotein folding, intracellular transport, secretion, and protection from proteolytic degradation. Our group has been describing the role of N-acetylglucosaminidase (NAGase) in fungal growth, exerted through participation in chitin metabolism and CW remodeling. In addition, by assessing yeast cells cultured with tunicamycin (TM), we determined that N-glycans play important roles in growth and morphogenesis of Pb yeasts and are required for the fungal NAGase function. In this study, we verify the influence of TM-mediated inhibition of N-linked glycosylation on a- and b-(1,3)-glucanase, as well as the a-(1,4)-amylase, produced by Pb yeast cells. The treatment of Pb with 15 mg TM/ml did not interfere with a- and b-(1,3)-glucanase production, secretion or on enzyme structure. The absence of N-glycans did not affect pH optimum (5.5) or temperature optimum (45 °C). Moreover, the fully- and under-glycosylated forms of the enzymes had similar Km and Vmax values. On the other hand, a-(1,4)-amylase demonstrated lower enzymatic activity when underglycosylated, although no difference was detected between the pH and temperature optimums of the two forms. Our results corroborates with the recent observation that a-(1,4)-amylase from Pb plays important roles on the fungal CW a-(1,3)-glucan biosynthesis. However, interestingly the Pbaglucan gene, that encode to a-(1,3)-glucanase, had its expression increased by 2.5-fold in Pb cells treated with TM when evaluated by qRT-PCR, suggesting an indirect influence of TM on CW glucan synthesis. Genes encoding to UPR (Unfolding Protein Response) and CW synthesis showed their expression increased, corroborating with our data. Analyses investigating the effect of N-glycans in mycelium cells are under way in our laboratory. Our results suggest that N-glycans do not play direct effect on a- and b-(1,3)-glucanase activity produced by yeasts cells but indirect effect by affecting a-(1,4)-amylase.

19. Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis. Vincent Bulone. Sch Biotech, Royal Inst Biotech (KTH), Stockholm, Sweden.

Cell wall polysaccharides play a central role in vital processes like the morphogenesis and growth of eukaryotic micro-organisms. Thus, the enzymes responsible for their biosynthesis represent potential targets of drugs that can be used to control diseases provoked by pathogenic species. One of the most important features that distinguish oomycetes from true fungi is their specific cell wall composition. The cell wall of oomycetes essentially consists of (1®3)-b-glucans, (1®6)-b-glucans and cellulose whereas chitin, a key cell wall component of fungi, occurs in minute amounts in the walls of some oomycete species only. Thus, the cell walls of oomycetes share structural features with both plants [cellulose; (1®3)-b-glucans] and true fungi [(1®3)-b-glucans, (1®6)-b-glucans and chitin in some cases]. However, as opposed to the fungal and plant carbohydrate synthases, the oomycete enzymes exhibit specific domain compositions that may reflect polyfunctionality. In addition to summarizing the major structural differences between oomycete and fungal cell walls, this presentation will compare the specific properties of the oomycete carbohydrate synthases with the properties of their fungal and plant counterparts, with particular emphasis on chitin, cellulose and (1®3)-b-glucan synthases. The significance of the association of these carbohydrate synthases with membrane microdomains similar to lipid rafts in animal cells will be discussed. In addition, distinguishing structural features within the oomycete class will be highlighted with the description of our recent classification of oomycete cell walls in three different major types. Genomic and proteomic analyses of selected oomycete and fungal species will be correlated with their cell wall structural features and the corresponding biosynthetic pathways.

20. Investigating the function of a putative chitin synthase from *Phytophthora infestans*. Stefan Klinter, Laura Grenville-Briggs, Hugo Mélida, Vincent Bulone. School of Biotechnology, Division of Glycoscience, Royal Institute of Technology (KTH), Stockholm, Sweden.

The oomycete *Phytophthora infestans* is a plant pathogen that causes potato late blight, a devastating disease associated with tremendous economic losses. In contrast to true fungi, oomycetes are traditionally described as cellulosic micro-organisms. Indeed, in addition to other b-glucans, cellulose is a major polysaccharide in the mycelial cell wall of *P. infestans* while chitin and other N-acetylglucosamine (GlcNAc)-based carbohydrates are absent from hyphal walls. However, a putative chitin synthase gene (*chs*) is present in the genome. Bioinformatic analysis identified the *C*-terminal region of the predicted protein to be highly similar to glycosyltransferase family 2 proteins, such as fungal chitin synthases, while the *N*-terminal domain is more divergent. Orthologous putative *chs* genes are present in all sequenced oomycete genomes and phylogenetic analysis shows the oomycete gene products form a new clade separate from the fungal lineage. The *P. infestans chs* transcript is highly abundant in older mycelium. However, no chitin synthase activity was detectable in microsomal fractions assayed with radioactively-labeled UDP-GlcNAc, the natural substrate of chitin synthase. Suprisingly, hyphal growth was severely retarded in the presence of low micromolar concentrations of the chitin synthase inhibitor nikkomycin Z, a structural analogue of UDP-GlcNAc. Microscopic analysis of nikkomycin Z-treated hyphae revealed frequent tip swelling and bursting. Similarly, transient RNA-mediated silencing of the *chs* gene resulted in severely reduced growth, and hyphae showed a hyper-branched morphology with swollen tips. As a first step to determine the precise function of the *P. infestans chs* gene, we have cloned and expressed it in *Saccharomyces cerevisiae*.

21. Deciphering cell wall structure and biosynthesis in oomycetes using carbohydrate analyses and plasma membrane proteomics. Hugo Melida[1], Vaibhav Srivastava[1], Erik Malm[1], J. Vladimir Sandoval-Sierra[2], Javier Dieguez-Uribeondo[2], Vincent Bulone[1]. 1) Division of Glycoscience, Royal Institute of Technology (KTH), Stockholm, Sweden; 2) Mycology Department, Royal Botanical Garden (CSIC), Madrid, Spain.

Some oomycete species are severe pathogens of economically important animals or plants. Proteins involved in cell wall metabolism represent excellent targets for disease control. The objective of our work was to determine the fine cell wall polysaccharide composition of selected species and identify the corresponding membrane-bound biosynthetic enzymes and other proteins involved in cell wall remodeling. In the first instance, we performed a detailed carbohydrate analysis of the mycelial cell walls of 11 oomycete species from 2 major orders, the Saprolegniales and Peronosporales. We then selected the fish pathogen *Saprolegnia parasitica* for in-depth proteomics analysis. Our results indicate the existence of 3 clearly different cell wall types. This

biochemical distinction is in agreement with the taxonomic grouping based on molecular markers of the species studied. The 3 cell wall types are distinguishable by their cellulose content and the fine structure of their 1,3-b-glucans. Furthermore, unique features were found in each case. Type I cell walls (e.g. *Phytophthora*) are devoid of N-acetylglucosamine (GlcNAc) but contain glucuronic acid and mannose; type II (e.g. *Achlya*, *Dictyuchus*, *Leptolegnia* and *Saprolegnia*) contain up to 5% GlcNAc and residues indicative of cross-links between cellulose and 1,3-b-glucans; type III (e.g. *Aphanomyces*) are characterized by the highest GlcNAc content (> 5%) and the occurrence of unusual carbohydrates that consist of 1,6-linked GlcNAc residues. Analysis of the recently sequenced genome of *S. parasitica* was combined with quantitative mass spectrometry-based proteomics (label-free and iTRAQ) to characterize the plasma membrane proteome of hyphal cells. This strategy allowed us to experimentally identify a total of 677 plasma membrane proteins, including several key cell wall polysaccharide synthases, e.g. cellulose, 1,3-b-glucan and chitin synthases, some of which are specifically enriched in plasma membrane microdomains similar to lipid rafts in animal cells.

22. Identification and characterization of the chitin synthase genes in the fish pathogen *Saprolegnia parasitica*. Elzbieta Rzeszutek, Sara Diaz, Vincent Bulone. School of Biotechnology, Division of Glycoscience, Royal Institute of Technology (KTH), Stockholm, Sweden.

The oomycete *Saprolegnia parasitica* is a fungus-like microorganism responsible for fish diseases and huge losses in aquaculture. The analysis of the cell wall composition of the microorganism and the characterization of key enzymes involved in cell wall biosynthesis may facilitate the identification of new target proteins for disease control. The cell wall of hyphal cells of *S. parasitica* consists mainly of cellulose, b-(1®3)- and b-(1®6) glucans, whereas chitin is present in minute amounts only. The main objective of this work was to test the effect of nikkomycin Z, a competitive inhibitor of chitin synthase (CHS), on the growth of *S. parasitica*. Genome mining allowed the identification of six different putative *chs* genes whose actual occurrence in the genomic DNA of the microorganism was confirmed by Southern blot analysis. The expression of the *chs* genes in the mycelium was analyzed using Real-Time PCR. The results revealed a higher expression level of four of the six genes while the two others exhibited undetectable levels of expression in the mycelium. This suggests that the latter genes are most likely primarily involved in chitin formation at a different developmental stage. The presence of nikkomycin Z increased the expression level of one of the genes, *chs3*, suggesting that the corresponding product is involved in forming the abnormal branching structures in the hyphae exposed to the inhibitor. The capacity of the mycelium to synthesize chitin was demonstrated by performing *in vitro* synthesis reactions using cell-free extracts. CHS activity was measured in intact cell membranes as well as in detergent-extract of membranes. The polysaccharide synthesized *in vitro* was characterized by enzymatic hydrolysis with a specific chitinase. Our data demonstrate that CHS represent promising targets of anti-oomycete drugs, even though the amount of chitin in the cell wall of *S. parasitica* does not exceed a few percent.

23. Role of Ccr4-mediated mRNA turnover in nucleotide/deoxynucleotide homeostasis and Amphotericin B susceptibility in Cryptococcus neoformans. D. Banerjee, J. Panepinto. Department of Microbiology and Immunology.University at Buffalo, SUNY, Buffalo, NY.

Ccr4 mediated deadenylation is the first and rate limiting step in eukaryotic mRNA decay. The end products of mRNA degradation are nucleoside monophosphates (NMPs) which are then converted to nucleotides (NDPs and NTPs) and deoxynucleotides (dNTPs) in downstream reactions. A C. neoformans degradation deficient ccr4D mutant exhibits replication stress sensitivity and stabilizes ribosomal protein (RP) transcripts during carbon starvation, suggesting that ccr4D mutant is deficient in intracellular nucleotide stores. Analysis of gene expression showed an up-regulation of the nucleotide synthesis machinery in ccr4D mutant even under unstressed conditions consistent with our hypothesis. Time-kill assays in the presence of mycophenolic acid (MPA), an inhibitor of guanine nucleotide de novo synthesis, showed a reduction in the viability of ccr4D mutant that was rescued by the addition of exogenous guanine, suggesting that the salvage pathway is indeed functional. These results suggest that the degradation of mRNA transcripts lead to the production of NMPs that replenish NTP/dNTP pools in C. neoformans during starvation stress. The fungicidal efficacy of Amphotericin B (AmpB) is enhanced by the use of Flucytosine, a pyrimidine analog, suggesting a synergy between AmpB and nucleotide deficiency for cryptococcosis treatment. We compared the sensitivity of wild type (H99), ccr4D mutant and H99-FOA strain (de novo mutant of pyrimidine synthesis) to a combination of AmpB and NTP/dNTP inhibitors. Both mutants exhibited higher sensitivity to AmpB which was unaltered by additional stressors. H99 exhibited an increased sensitivity to the combination of AmpB with both NTP and dNTP inhibitors, compared to AmpB alone. Taken together, these data suggest that nucleotide depletion, either by a pharmacologic agent or a mutation predisposes the cells to enhanced AmpB mediated cell death.Thus our overall hypothesis is that Ccr4 mediated mRNA turnover results in the maintenance of intracellular NTP/dNTP pools to promote growth, virulence, stress tolerance and also modulates Amp B susceptibility in C. neoformans. Results from these studies will identify a novel role of the mRNA degradation machinery in C. neoformans pathogenesis and stress tolerance and also aid in the identification of new anti-cryptococcal drug targets.

24. *WITHDRAWN*

25. Blue light induce Cordyceps militaris fruiting body formation and cordycepin production. Chun-Hsiang Yang[1], Shun-Kuo Sun[2], Su-Der Chen[1]. 1) Biotechnology and Animal science, National Ilan University, Ilan, Taiwan; 2) Bionin Biotechnology, INC.

Cordyceps militaris is a very important fungal medicine in Chinese. The fruiting body of Cordyceps militaris has been described by many researchers containing biological activies, such as being able to inhibit cell proliferation, provide anti-ageing activity, inhibit protein synthesis and lowing cardiovascular risk. Cordyceps militaris has been grown and harvested by many Chinese people, and were able to obtain its fruiting body with orange collar and bar shape. Solid cultivation as carried out 3 to 4 days after mycelium seeding from liquid culture, then fruiting body formation can been induced by light(12 hours per day). In this research, the light-inducing mechanism of fruiting body formation was studied. The results showed the fruiting body was induced by blue light but not red light. Cordycepin, the most important compound with medical potential of Cordyceps militaris, is mainly stored in fruiting body, rather than in mycelium from liquid culture. Cordycepin production depends on various factors, including: wave length of light and culture in solid or liquid. The results also showed the relationship between cordycepin production and blue light sensor in Cordyceps militaris, which might contain LOV domain.

26. Insight into alkaloid diversity of the epichloae, protective symbionts of grasses. Carolyn A. Young[1], Nikki D. Charlton[1], Johanna E. Takach[1], Ginger A. Swoboda[1], Bradley A. Hall[1], Kelly D. Craven[2], Christopher L. Schardl[3]. 1) Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK; 2) Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK; 3) Plant Pathology, University of Kentucky, Lexington, KY.

Cool season grasses from the subfamily Pooideae often form symbiotic associations with fungal endophytes known collectively as the epichloae (*Epichloë* and *Neotyphodium* species). The epichloae consist of both sexual (nonhybrid) and asexual (hybrid and nonhybrid) species that can produce the bioactive anti-herbivore compounds, ergot alkaloids, indole-diterpenes, lolines and peramine. Epichloae can exhibit considerable chemotypic diversity within the pathways of these four alkaloid classes as well as the combination of alkaloids that can be produced by an individual, and as such, may equate to fitness benefits for the host. The current genome sequencing efforts, whereby at least 10 epichloae have been sequenced, now allows us to develop simple

approaches to rapidly screen large cool-season grass populations to identify endophyte diversity. Molecular analyses of endophyte genetic traits from among and between host populations allow us to explore resident endophyte incidence and diversity present in single host species. PCR with genomic DNA extracted from individual plants (seeds or tillers) is used to determine endophyte incidence within a line and to predict alkaloid chemotypes at the *EAS* (ergot alkaloids), *LOL* (lolines), *IDT/LTM* (indole-diterpenes) and *PER* (peramine) loci. The presence or absence of genes at each locus can be used to predict the likely pathway end product for a given endophyte-infected plant line. Phylogenetic analyses of housekeeping and mating-type genes are used to infer hybrid versus nonhybrid origins as well as hybrid ancestral progenitors. Sequence analyses of alkaloid genes encoding key pathway steps provide allele copy number and can be used to determine progenitor origins to further support the phylogenetic relationships. Grass collections across multiple host tribes have recently been evaluated and considerable endophyte chemotypic diversity was identified. Multiple endophyte species were able to independently associate with some grass host species and often both hybrid and nonhybrid endophytes could be found within a population. In many cases, chemotypic diversity of the hybrids may have arisen from independent hybridization events and as such, this alkaloid diversity likely translates into differences in fitness and persistence of the host.

27. Extracellular polysaccharide degrading capabilities of various *Agaricus bisporus* strains during compost cultivation. A. Patyshakuliyeva, J. Yuzon, R. P. de Vries. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

In the temperate forests of North America and Europe, basidiomycetes such as *Agaricus bisporus* are renowned for their ecological significance in the cycling of carbon from dead plant matter. *A. bisporus* is also the most widely produced mushroom in the world and has been cultivated for centuries. However, little is known about the interaction between *A. bisporus* and its most preferred substrate, composted plant matter. In this study, a wide array of extracellular polysaccharide degrading enzymes was studied under semi-commercial conditions to understand the carbon nutritive needs of the fungi. Various time points were sampled from filling of the beds, vegetative growth and development and maturation of fruiting bodies. Clear correlations in the enzymatic activities were observed from different stages of development of *A. bisporus* between compost, casing layer and fruiting bodies. This could suggest that vegetative mycelia and the fruiting body divide their metabolic roles as vegetative mycelium of *A. bisporus* provides nutrients for the growth of fruiting bodies, while fruiting bodies aims on reproduction. This was also confirmed by identification of the expression of genes encoding plant and fungal polysaccharide modifying enzymes in compost, casing layer and fruiting bodies.

28. Reconstruction of the rubrofusarin biosynthetic pathway in *Saccharomyces cerevisiae*. Rasmus J N Frandsen[1], Peter Rugbjerg[1], Michael Naesby[2], Uffe H Mortensen[1]. 1) Systems Biology - CMB, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Evolva SA, Duggingerstrasse 23. CH-4153 Reinach, Switzerland.

The aromatic heptaketide rubrofusarin is a common core substructure of several fungal pigments, including rubrofusarin B, aurofusarin, nigerone, nigerasperone A, chaetochromin, ustilaginoidin and parasperone A. Compounds that are produced by a wide variety of different filamentous fungi such as *Fusarium graminearum, Aspergillus niger, Aspergillus parasiticus, Chaetomium gracile* and *Ustilaginoidea virens*. Previous reverse genetics analysis of the aurofusarin biosynthetic pathway, by targeted gene replacement in *F. graminearum* (*Fg*), has resulted in the formulation of a six step biosynthetic pathway that includes rubrofusarin as an intermediate. In the current study we have used heterologous expression in *Saccharomyces cerevisiae* to test whether all the enzymes required for biosynthesis of rubrofusarin have been identified. Successful reconstruction of the rubrofusarin pathway is dependent on the heterologous co-expression of four genes: the *Fg* polyketide synthase *PKS12*, the *Fg* dehydratase *aurZ*, the *Fg* O-methyltransferase *aurJ* and the *Aspergillus fumigatus* phosphopantetheine transferase *npgA*. To eliminate potential problems with intron splicing of the fungal genes in *S. cerevisiae* the required coding sequences were *de novo* synthetized in codon optimized versions. The four genes were expressed individually from four different single copy plasmids, each with a unique auxotrophic marker. Co-expression of the codon optimized version of *PKS12* with *npgA* did not result in production of any new metabolites. However, surprisingly co-expression of a cDNA version of *PKS12*, assembled from gDNA by USER-fusion, resulted in production of the expected product YWA1. Additional co-expression of the codon optimized dehydratase encoding *aurZ* gene lead to production of nor-rubrofusarin, and subsequent introduction of the O-methyltransefase gene *aurJ* yielded rubrofusarin. These results support the previously proposed biosynthetic route for the formation of rubrofusarin in *F. graminearum*. The utilized bottom-up approach shows that formation of rubrofusarin is dependent only on the combined action of PKS12, AurZ and AurJ in *F. graminearum*, and likely also in other fungal species that produce compounds with a rubrofusarin core. The latter is further supported by sequence base homology searches in the available relevant fungal genome sequences.

29. Expression and purification of hydrophobin fusion proteins targeted to intracellular protein bodies in *T. reesei* . Nina K. Aro, Marika Vitikainen, Jussi Joensuu, Eero Mustalahti, Markku Saloheimo. Biotechnology, VTT Technical Research Centre, 02044 VTT, VTT, Finland.

Recombinant protein production is a fast growing market area. The need for novel production platforms is growing together with the number of new applications for recombinant proteins. The ascomycete *T. reesei* is an excellent producer of hydrolytic enzymes. However, heterologous protein production in *T. reesei* is often suffering from low product yields due to protease degradation and inefficiency in heterologous protein secretion. We have previously demonstrated a novel recombinant protein production system for *T. reesei* using GFP as a model protein. This system uses hydrophobin, a small and amphipathic fungal protein, as a fusion tag for purification and ER retention signal for targeting the produced protein to intracellular protein bodies. The GFP-HFBI fusion protein can be extracted from total protein lysate by aqueous two-phase separation system. We have now further optimised the expression for GFP-HFBI fusion and demonstrated the applicability of this production concept for two additional proteins, glucose oxidase (GOX) and tissue plasminogen activator (tPA). Effect of C- and N-terminal hydrophobin fusion on productivity and extraction in two-phase separation system will be discussed. The new production concept is aiming at widening the spectrum of recombinant proteins that can be produced efficiently in *T. reesei*.

30. Metabolic adaptations in *Phytophthora infestans* and the role of a phosphagen kinase system in energy metabolism. Meenakshi Kagda, Howard Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521.

Nutrient acquisition and metabolic adaptation to host-derived nutrients is an important aspect of pathogen biology. An understanding of the metabolic adaptations made by *Phytophthora infestans*, an important pathogen of potato and tomato, to optimize nutrient uptake from diverse host tissues and within the microenvironments of the host will lead to a better understanding of host-pathogen relationships. In order to study metabolic adaptations of *P. infestans*, transcriptional profiling and live cell imaging using promoter-fluorescent protein fusions will be used. Preliminary results demonstrated the differential gene expression of many metabolic genes of *P. infestans* grown on different natural hosts and that grown on rich media. The next step involves answering the question: Are some metabolic genes expressed in a stage-specific or time-dependent manner? In addition, the role of enzymes involved in energy homeostasis and metabolite channeling are being studied. The roles of two such genes encoding putative creatine kinases are being elucidated using subcellular localization, substrate utilization and loss of function studies.

31. Platforms for secondary metabolite analysis in filamentous fungi. Uffe H. Mortensen[1], Jakob B. Nielsen[1], Diana C. Anyaogu[1], Dorte K. Holm[1], Lene M. Petersen[1], Morten T. Nielsen[1], Mikael S. Joergensen[1,2], Kristian F. Nielsen[1], Pia F. Johannesen[2], Dominique A. Skovlund[2], Thomas O. Larsen[1]. 1) DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Department 463, Fungal Gene Technology, Novozymes, Denmark.

We are developing versatile methods that allows for rapid and simple genetic manipulation of filamentous fungi. Currently, we use our methods for elucidation of pathways for secondary metabolite production in a number of different species. The platform includes simple systems for gene targeting and defined expression platforms for pathway reconstitution. Alternatively, if few or no genetic tools are available for the fungus, we use AMA1 based plasmids for transformation. All DNA handling prior to fungal transformation is based on assembly by efficient USER cloning that allows for many DNA fragments to be merged in a single cloning step. Examples of pathway reconstitution will be presented including functional transfer of the entire geodin producing gene cluster from *Aspergillus terreus* into *A. nidulans*. In an attempt to map the first intermediates of polyketide pathways in a fungal species, we have individually expressed all PKS genes from *A. niger* as a starting point for pathway elucidation. Using this approach we identified a PKS gene responsible for production of 6-MSA. Next, we individually deleted all genes in the corresponding gene cluster in *A. niger* to further map the pathway. Theses analyses suggest that 6-MSA is a precursor of Yanuthone D/E. In a similar study, we have identified a related PKS gene in *A. aculeatus* that also produces 6-MSA when expressed in *A. nidulans*. The corresponding gene cluster in *A. aculeatus* contains a gene encoding a transcription factor. Using our AMA1 based expression system, this gene has been overexpressed in *A.aculeatus*. As a result a new 6-MSA based compound has been identified. Lastly, using a knock-in/knock-out platform in *Trichoderma reesei* we use the same principles to uncover a gene cluster that is responsible for a very complex family of sorbicillinoids.

32. Transcriptional analysis of oxalate degradation in the white rot basidiomycete *Dichomitus squalens*. Miia R. Mäkelä, Johanna Rytioja, Outi-Maaria Sietiö, Sari Timonen, Annele Hatakka, Kristiina Hildén. Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

Basidiomycetous white rot fungi are the most efficient degraders of lignocellulose with a unique ability to mineralize the recalcitrant lignin polymer. Lignocellulose decay involves a complex enzymatic system, but is also suggested to be promoted by the fungal secretion of oxalic acid. White rot fungi synthesize oxalate as a metabolic waste compound and typically secrete it to their environment in millimolar quantities. As oxalate is a toxic compound, regulation of its intra- and extracellular concentration is extremely crucial for fungi and also for lignocellulose degradation since high oxalate levels are shown to inhibit the decomposition reactions. Therefore, specific oxalate-converting enzymes, namely oxalate decarboxylases (ODCs) that work in conjunction with formate-degrading formate dehydrogenases (FDHs), are recognized as key fungal enzymes in lignocellulose decay. *Dichomitus squalens* is a white rot fungus that degrades effectively all the wood polymers, i.e. cellulose, hemicelluloses and lignin, and secretes oxalic acid during its growth on wood. The genome of *D. squalens* harbours 5 putative ODC and 3 putative FDH encoding genes, while these numbers differ in other fungi based on comparative genomics. In order to enlighten the roles of the multiple oxalic-acid catabolising enzymes of *D. squalens*, the expression of the *odc* and *fdh* genes was followed with quantitative real-time RT-PCR when the fungus was grown on its natural substrate, i.e. Norway spruce (*Picea abies*) wood. In addition, the effect of organic acid (oxalic acid) and inorganic acid (HCl) supplementation on the relative transcript levels of the oxalate-catabolizing genes was examined in the submerged liquid cultures of *D. squalens*. The results show for the first time the sequential action of ODC and FDH at the transcript level in a white rot fungal species. The constitutive expression of *odc1* suggests the pivotal role of the corresponding enzyme during the growth of *D. squalens* on wood. In addition, the strong upregulation of the transcription of *odc2* in oxalic-acid amended cultures indicates the distinct roles of individual ODC isoenzymes.

33. Creation of temperature-influenced hyphal growth mutants in a basidiomycete fungus through the use of UV mutagenesis. Stephen J. Horton, Carly Wender, Suhasini Padhi. Dept Biological Sci, Union Col, Schenectady, NY.

Filamentous fungi have been used extensively in industry for decades, most prominently for the purposes of protein expression. An emerging technology is the use of fungi in the production of ecologically-friendly materials used in packaging and insulation, materials presently manufactured using non-renewable petroleum-based technologies. The growth characteristics of the mycelia used in these manufacturing processes play a pivotal role in the properties of the final product. We decided to utilize the classical approach of UV mutagenesis to create new strains of a basidiomycete fungus that would potentially have growth characteristics more suited than the wild type to particular industrial applications. One example of this would be the production of fungal strains with a wider temperature spectrum for growth, a factor applicable to the industrial reality of the fluctuating ambient temperature found in non-laboratory conditions. Protoplasts from our wild-type strain were subjected to 70,000 microjoules/cm2 of UV irradiation at a wavelength of 254 nm. Survivors were allowed to recover overnight at 28°C, and then plated at a selective temperature of 40°C. Out of an estimated 6400 protoplasts irradiated, we observed 48 colonies of various sizes after 11 days growth at the selective temperature of 40°C. This temperature was chosen because it approaches the maximum permissible growth temperature for this fungus. We further characterized 22 of these presumed mutants and were able to sort them into broad categories based upon their growth rates over the range of 28°C to 37°C. The categories were: (1) strains that grew well at the normal laboratory temperature (28°C), but less well at elevated temperatures (33.5°C and 37°C), (2) strains that grew best at elevated temperature (33.5°C), and (3) strains that grew at relatively similar rates at all three temperatures. The morphology of the hyphae (density, branching pattern) was also found to differ between the growth mutants. Selected mutants will be analyzed by both RNA seq and genomic sequencing approaches in an effort to identify any common genes that may have been altered as a result of the mutagenesis regime.

34. Functional Analysis of a Novel Diaminopimelate Decarboxylase from the Oomycete Saprolegnia parasitica. Lingxiao ge[1], Josie Hug[1], Stan Oome[2], Paul Morris[1]. 1) Biological Sci, Bowling Green State Univ, Bowling Green, OH; 2) Plant-Microbe Interactions, Utrecht University, The Netherlands.

In bacteria and plants, the lysine precursor L, L-diaminopimelate (DAP) is first converted to meso-diaminopimelate by an epimerase. Then meso-DAP is converted to lysine by a DAP decarboxylase. Comparative analysis of seven sequenced oomycete genomes, revealed that only Saprolegnia parasitica contains a predicted epimerase. Sequence homology in all of the predicted DAP decarboxylases in oomycetes is strongly conserved, suggesting that these proteins have similar biochemical activity. The oomycete DAP gene appears to have been acquired by horizontal transfer from Archaea sp. Notably, these particular Archaea sp. have all the genes needed to synthesize lysine, except for epimerase. Thus we postulated that the oomycete DAP might be a novel enzyme capable of converting L, L-DAP directly to lysine. To test this hypothesis, we codon-optimized the DAP gene from S. parasitica and expressed it in an E. coli DAP mutant. Complementation assays of the mutant expressing the S parasitica gene in lysine-minus media indicate that the gene functions as a DAP decarboxylase. To determine the substrate specificities of the S. parasitica DAP gene, we have developed an HPLC method to separate the D, L, and meso isomers of chemically synthesized DAP. Authentic L, L-DAP has also been purified from the culture filtrates of an E coli epimerase mutant. Functional assays of the affinity-purified protein will enable us to characterize the substrate specificities of the oomycete enzyme. If the S. parasitica DAP enzyme can utilize L, L-DAP as a substrate, then the retention of epimerase in this genome may indicate that meso-DAP is incorporated into the cell wall of this group of organisms.

35. Living on Air?: *Ustilago maydis* cells grow without being provided nitrogen in their growth media. Michael H Perlin, Michael Cooper. Dept Biol and Program on Disease Evolution, Univ Louisville, Louisville, KY, USA.

Nitrogen is an essential nutrient for all living creatures. Ammonium is one of the most efficiently used and thus preferred, sources of nitrogen. As with other dimorphic fungi, yeast-like cells of *Ustilago maydis*, the fungal pathogen of maize, switch to filamentous growth when starved for nitrogen/ammonium. *U. maydis* carries two genes, *ump*1 and *ump2*, encoding ammonium transporters that facilitate both uptake of ammonium and the filamentous response to its absence. While no obvious phenotype is observed when *ump1* is deleted, cells without *ump2* are unable to filament in response to low ammonium, although they can still grow. Surprisingly, *ump1ump2* double mutants can also grow on low ammonium. More amazing still, both wild type and mutant cells continue to grow, even after strenuous efforts were made to remove all nitrogen sources from their growth media. To investigate these unusual observations further, we grew wild type and mutant cells in the absence or presence of added nitrogen, as ammonium or supplied as ^{15}N gas. Septum bottles with rich, low ammonium and no ammonium media were inoculated with rinsed overnight wild type and mutant cells, injected with +0.1% $^{15}N_2$ and were then incubated for seven days. The resulting biomass was sampled for microscopic examination, collected by filtration, dried and loaded into tin sample capsules for $d^{15}N$ analysis by the Stable Isotope Research Unit at Oregon State University. The wild type cells under rich, minimal and no ammonium conditions had mean $d^{15}N$ ratios of 0.7, 10.8 and 45.2, respectively, while the mutant cells had mean $d^{15}N$ ratios of 3.29, 49.5 and 134.8, respectively, for these growth conditions. This indicated significant incorporation of the ^{15}N tracer from the injected gas into the cellular biomass. We are currently investigating additional candidate genes that may play a role in this novel capability by a fungus.

36. Saprotrophic metabolism of the White-Nose Syndrome fungus *Geomyces destructans* in bat hibernacula. Hannah Reynolds[1], Tom Ingersoll[2], Hazel Barton[1]. 1) Department of Biology University of Akron Akron, OH 44325; 2) National Institute for Mathematical and Biological Synthesis (NIMBioS) University of Tennessee Knoxville, TN 37996.

Geomyces destructans (Myxotrichaceae, Leotiomycetes), an emerging epizootic disease of hibernating bats in North America has arisen from a predominately saprotrophic genus. We have isolated multiple, non-infectious *Geomyces* species from cave surfaces and healthy bats for physiological and genetic comparison with *G. destructans* to better understand its disease ecology. In particular, we are interested in 1) whether *G. destructans* retains saprotrophic ability, acting as a facultative rather than an obligate pathogen and 2) identifying the microhabitats that support *Geomyces* and presumably *G. destructans* growth. Identifying an environmental niche for *G. destructans* would aid in understanding future disease ecology. Comparative genomics indicates the presence of multiple enzymes involved in saprotrophic metabolism, including endoglucanases, b-glucosidases and chitinases, while in vitro saprotrophic assays demonstrate similar cellulase and lipase functions in both pathogenic and non-pathogenic *Geomyces.* To understand the native microbial habitats that might inhibit or promote *G. destructans* growth we used molecular phylogenetic analyses of environmental fungal ITS sequences to examine both the overall fungal diversity and the diversity of *Geomyces* in multiple cave microhabitats. Knowledge of the specific habitat of *G. destructans* will allow us to determine the likelihood for saprophytic growth within caves and estimate the role that subsidies can play in disease ecology. Indeed, disease modeling indicates that an environmental subsidy for the growth of *G. destructans* increases the likelihood of bat host extinction events.

37. Cellulose acting enzymes of the white-rot fungus *Dichomitus squalens*: expression of the genes and characterization of the enzymes. Johanna Rytioja, Aila Mettälä, Kristiina Hildén, Annele Hatakka, Miia Mäkelä. Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

Plant biomass is a diverse raw material that has great potential to be exploited e.g. in second generation biorefinery applications. In order to overcome the economic and technological thresholds in biomass utilization, novel cellulose attacking enzymes and optimal enzyme mixtures are needed. The synergistic effect of cellulose hydrolyzing enzymes, namely endoglucanases (E.C. 3.2.1.4), cellobiohydrolases (CBH, E.C. 3.2.1.91) and b-glucosidases (E.C. 3.2.1.21), during cellulose degradation is a well-defined phenomenon, which has also been reported for cellulose oxidizing enzymes. The fungal produced oxidative enzymes related to cellulose degradation include cellobiose dehydrogenases (CDH, E.C. 1.1.99.18) and the proteins of glycoside hydrolase (GH) family 61 (www.cazy.org).

Basidiomycetous white-rot fungi are able to efficiently degrade all the wood polymers, i.e. cellulose, hemicelluloses and lignin. Their lignin-modifying oxidoreductases (peroxidases and laccases) are rather well characterized, whereas their cellulose acting enzymes (CAZymes) have so far gained less attention. In the large screening of hydrolytic enzymes of basidiomycetous fungi from the Fungal Biotechnology Culture Collection (FBCC, University of Helsinki), the white-rot fungus *Dichomitus squalens* was found to produce high cellulolytic activity and appeared as a promising source of novel CAZymes.

In this work, the expression of selected hydrolytic and oxidative CAZyme encoding genes (*cdh*, four *cbh*s, five putative *gh61*s) was followed with quantitative real-time RT-PCR during the growth of *D. squalens* on solid Norway spruce (*Picea abies*) wood and in semi-solid microcrystalline cellulose (Avicel) -peptone liquid medium. The enzymatic activities of cellulases and xylanase as well as lignin-modifying oxidoreductases were measured from the semi-solid cultures. In addition, CBHI and CDH enzymes of *D. squalens* were purified and characterized.

38. Metabolomics of growth and type B trichothecenes production in *Fusarium graminearum*. L. Legoahec[1], V. Atanasova-Penichon[1], N. Ponts[1], C. Deborde[2,3], M. Maucourt[3,4], S. Bernillon[2,3], A. Moing[2,3], F. Richard-Forget[1]. 1) 1INRA, UR1264 MycSA, 71 avenue Edouard Bourlaux, BP81, F-33140 Villenave d'Ornon, France; 2) INRA, UMR1332 Fruit Biology and Pathology, 71 avenue Edouard Bourlaux, BP81, F-33140 Villenave d'Ornon, France; 3) Metabolome Facility of Bordeaux Functional Genomics Center, IBVM Centre INRA de Bordeaux, F-33140 Villenave d'Ornon, France; 4) Univ. Bordeaux, UMR1332 Fruit Biology and Pathology, Centre INRA de Bordeaux, F-33140 Villenave d'Ornon, France.

The plant fungal pathogen *Fusarium graminearum* can produce type B trichothecenes, a family of sesquiterpene molecules with toxic properties upon human or animal ingestion. Deoxynivalenol, or DON, and its acetylated forms belong to this family of secondary metabolites and are frequent contaminants of cereals worldwide. The biosynthesis of trichothecenes initiates with the condensation of two molecules of farnesyl pyrophosphate, at the end of the mevalonate pathway in *Fusarium*, and is under the control of various factors such as the redox parameters of the environment or the carbon source. For example, supplementing liquid submerged cultures of *F. graminearum* with caffeic acid, a phenolic acid with known antioxidant properties, reduces the accumulation of DON and its acetylated forms in the medium. Such a result, however, gives a partial glimpse of the effect of phenolic acids, from the trichothecene production point of view only. The present study analyzes *F. graminearum* metabolome in conditions when DON and its acetylated forms are produced. Liquid chromatography coupled with mass spectrometry and proton nuclear magnetic resonance were used to characterize the metabolites produced by the fungus, secreted in the culture medium or not, over the course of 14 days. Fifty-two polar and semi-polar metabolites were identified in the culture medium, *i.e.,* the exo-metabolites, and/or in the mycelium, *i.e.,* the endo-metabolites, comprising amino acids and derivatives, sugars, polyketides, and terpenes including trichothecenes and DON precursors. Sample composition varied over time in terms of primary metabolites as well as secondary metabolites. Data analysis further revealed correlations, positive or negative, between metabolic pathways. In the presence of caffeic acid, metabolomic profiles were modified, counting those resulting from primary metabolism even though fungal biomass production was not affected by the treatment. Several metabolites affected by the treatment were identified for both the exo- and endo-metabolome, in particular DON and its precursors. For the first time, these results expose a unique outlook of a hidden aspect of *Fusarium*'s response to antioxidant treatment.

39. Diversity of telomeric sequences and telomerase RNA structures within Ascomycetes. Xiaodong Qi, Yang Li, Dustin P. Rand, Julian J-L Chen. Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ, 85287-1604.

Telomeres are specialized DNA-protein complexes that cap chromosome ends. Telomeric DNA is composed of repetitive short sequences synthesized by telomerase, an RNA-containing DNA polymerase. The integral telomerase RNA (TER) contains telomerase provides a short template for telomeric DNA synthesis and two highly conserved structural elements essential for enzymatic function. Fungal telomerase from budding and fission yeasts has been studied extensively. We have recently developed *Neurospora crassa* as a new fungal model organism for telomere and telomerase studies, and have identified TER structural domains highly conserved in vertebrate and Pezizomycotina, but not in budding yeasts (Qi et al. 2012). *N. crassa* telomerase processively synthesizes the (TTAGGG)n telomere repeats, an attribute conserved in vertebrate but not yeast telomerases. In contrast, both budding and fission yeast telomerases synthesize irregular telomere repeats non-processively. Two structural elements of TER, the template-pseudoknot and the three-way junction (TWJ) domain, are conserved in vertebrates, Pezizomycotina as well as Taphrinomycotina. Both of these elements are necessary for telomerase activity *in vitro* for Pezizomycotina and Taphrinomycotina telomerases, while the TWJ is dispensable for budding yeast telomerase. Furthermore, spliceosome-mediated TER 3'-end processing is conserved in Pezizomycotina and Taphrinomycotina, but not in budding yeasts. In comparison, the budding yeast (e.g. *S. cerevisiae*) TER employs a nuclease-mediated mechanism for the 3' end processing. Our results indicate that Pezizomycotina telomerase preserved ancestral features that budding and fission yeast species lost during evolution and supports *N. crassa* as an excellent model for the study of telomere and telomerase. (Reference: Qi, X., Y. Li, S. Honda, S. Hoffmann, M. Marz. A. Mosig, J.D. Podlevsky, P.F. Stadler, E. Selker and J.J.-L. Chen (2012) The common ancestral core of vertebrate and fungal telomerase RNAs. Nucleic Acids Research 40: doi:10.1093/nar/gks980.).

40. Characterizing a putative three-step formaldehyde oxidation pathway in *Neurospora crassa*. Ethan Addicott[1], Kolea Zimmerman[2], Anne Pringle[2]. 1) Faculty of Arts and Sciences, Harvard College, Harvard University, Cambridge, MA; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Using bioinformatic analyses, we identified 13 *Neurospora* genes that code for putative secreted-proteins. One of these proteins, NCU01056 - a proposed S-(hydroxymethyl)glutathione synthase, is implicated in a highly conserved formaldehyde oxidation pathway involving two other genes, NCU06652 - an NAD and GSH dependent formaldehyde dehydrogenase and NCU0173- an S-formylglutathione hydrolase. Knockout strains for the three genes in this pathway were obtained from the FGSC and confirmed by PCR. We conducted standard phenotypic assays on the three knock-outs and WT controls, including growth morphology, growth rate, and mating ability. Additionally, growth in the presence of methanol, the compound just upstream of formaldehyde in the pathway, was tested by biomass and flow cytometry. Two key observations were made: (1) NCU06652 knockouts showed significant growth defects compared to the WT (2) Knockouts for NCU01056 (hypothesized to be upstream of the critical enzyme) showed increased pigmentation on SC media (3) NCU6652 knockouts germinated significantly slower than other strains in the presence of methanol compared to a control treatment. The data suggest NCU06652 is involved in the critical oxidation step of the pathway and that the absence of NCU01056 may induce stress, which points to its role in the formation of a formaldehyde-glutathione complex, immediately upstream of NCU06652. The fact that NCU01056 codes a secreted protein may suggest that *N. crassa* may detoxify formaldehyde extracellularly or in membrane-bound vesicles. Further exploration will involve determining a dose-response curve for formaldehyde, confirming the localization of the proteins, and investigating the GSH balance in each of the strains.

41. Nitrate assimilation in *Neurospora crassa*. Oleg Agafonov, Tina Marie Monge Are, Peter Ruoff. Centre for Organelle Research, University of Stavanger, Stavanger, Norway.

Nitrogen is one of the essential components for a variety of cellular elements. Regulation of nitrogen assimilation can be critical for the evolutionary advantage of an organism and it has been extensively studied in filamentous fungi *Neurospora crassa*. Nitrate is an important source of inorganic nitrogen for *N. crassa*, but it is not utilized unless favored nitrogen sources such as ammonium, glutamine or glutamate are absent in the environment. It was shown that nitrate is transported into the cell by high affinity transporter, NIT10, where it is stepwise reduced, first, by nitrate reductase, NIT3 to nitrite, and then by nitrite reductase, NIT6 to ammonia, which is then converted to organic nitrogen in a form of glutamate, making it available for further utilization by the cell.

Although biochemical pathways of nitrate assimilation have been extensively studied, there is a certain disagreement in literature about the requirement of functional nitrate reductase activity for nitrate uptake. In the paper by Schloemer and Garrett, 1974, it was shown that nitrate transport is not dependent upon nitrate reduction. However, later Unkles et. al., 2004, concluded that functional nitrate reductase is required for the nitrate accumulation in *Neurospora crassa*.

The goal of this work was to investigate nitrate assimilation and involvement of nitrate reductase in this process in *N. crassa*. Nitrate disappearance from

the media and weight of the mycelium were measured in *nit-10* (KO) and *nit-3* (KO) strains in comparison to a wild type strain. The *nit-10* (KO) mutant was not able to grow on nitrate as a sole nitrogen source, and no nitrate depletion from the media was observed. Therefore, it was concluded that NIT10 is the only active transporter. The accumulation of nitrate in the mycelium was measured and it was found that in the *nit-3* (KO) mutant it was 9 times higher and in the *nit-10* (KO) mutant 3 times higher than in the wild type strain. Therefore, we concluded that nitrate reductase is not required for the nitrate transport.

42. Protein kinases affecting glycogen accumulation and likely regulating the glycogen synthase phosphorylation status in *Neurospora crassa*. T. Candido[1], A. P. Felício[2], R. Gonçalves[1], F. Cupertino[1], F. Freitas[1], M. C. Bertolini[1]. 1) UNESP - IQ - Araraquara, Araraquara, SP., Brazil; 2) Departamento de Genética e Evolução, UFSCar, São Carlos, SP, Brazil.

The ability to sense and respond appropriately to environmental changes is required for all living organisms and reversible phosphorylation of proteins mediated by protein kinases plays a key role in this aspect. In this work we describe the results of a screen aimed to identify protein kinases regulating glycogen metabolism in *Neurospora crassa*. The glycogen synthase (GS) and glycogen phosphorylase (GP), the regulatory enzymes in glycogen synthesis and degradation processes, respectively, are highly regulated by phosphorylation, however the protein quinases that phosphorylate them in *N. crassa* have not been identified. In this work, a set of mutant strains individually knocked-out in genes encoding proteins kinases was used. The glycogen levels were quantified under normal growth temperature (30°C) and under heat stress (45°C). From 84 mutant strains, 37 strains presented glycogen accumulation profile different from the wild-type strain suggesting that the missing protein kinase is implicated in glycogen metabolism control. Among the protein kinases selected most are Ser/Thr protein kinases, and it is important to mention proteins already characterized as regulators of glycogen metabolism, such as the *Saccharomyces cerevisiae* Pho85 and Snif1 proteins. The GSN activity was quantified in the selected strains grown under normal temperature (30°C) and under heat stress (45°C) in the presence and absence of the allosteric activator glucose-6-phosphate (G6P). The ratio -/+ G6P is considered as an index of phosphorylation, lower levels correlating with higher phosphorylation. Some protein kinases were implicated in glycogen metabolism control by likely influencing the GSN phosphorylation status. The GSN phosphorylation profile in the mutant strains were analyzed in 2D-PAGE followed by Western blot using polyclonal GSN antibody. Some mutant strains showed phosphorylation profile different from the wild-type strain and the results revealed putative proteins kinases not yet described as able to phosphorylate GSN. The expression of glycogen synthase (*gsn*) and glycogen phosphorylase (*gpn*) genes was analyzed by qRT-PCR in the mutant strains and the results showed that some protein kinases regulate the expression of both genes. Supported by FAPESP and CNPq.

43. Endogenous ergothioneine is required for wild type levels of *Neurospora crassa* conidiogenesis and conidial survival, but does not protect against uv-induced kill or mutagenesis. Lynn Epstein, Marco Bello, John Mogannam. Plant Pathology, University of California, Davis, CA. 95616-8680.

Ergothioneine (EGT) is a histidine derivative that apparently is only synthesized by fungi (except in the Saccharomycotina), and by some bacteria in the Cyanophyta and Actinomycetales. Although plants and animals do not synthesize EGT, they acquire it from the environment; EGT is concentrated in animal cells with an EGT-specific transporter. Bello et al. (2012, Fungal Genet Biol 49:160) showed that the concentration of EGT is 5x greater in *Neurospora crassa* conidia than in mycelia, and that growth of strain NcDEgt-1 with a knockout in gene NCU04343 is indistinguishable from the wild type. To investigate the function of EGT, wild type (Egt+) and NcDEgt-1 were crossed and six Egt+ and six Egt- sib strains were analyzed. Compared to the Egt+ sibs, Egt- sibs had a highly significant reduction (59 $\pm$ 6%, $\pm$ SE) in the number of conidia produced on Vogel's agar; the detransformed mean of the Egt- sibs was 1.5×10^5 conidia/cm^2 with a detransformed 95% confidence interval (CI_{95}) from 1.2×10^5 to 1.8×10^5 conidia/cm^2 whereas the Egt+ sibs had a mean of 3.6×10^5 conidia/cm^2 and a CI_{95} from 2.9×10^5 to 4.6×10^5 conidia/cm^2. The concentration of EGT in wild type conidia did not increase with increasing exposure to light during conidiogenesis. Seven-day-old conidia were stored at 30 °C at 97% and 51% relative humidity (RH) for a time course to either 17 or 98 days, respectively. Life expectancies (LE) were calculated from logistic curves fitted to percentage germination as a function of days in storage in two trials. At 97% RH, Egt+ sibs had a LE = 11.0 $\pm$ 0.2 days whereas Egt- sibs had a highly significantly lower LE = 8.4 $\pm$ 0.2 days, a 23 $\pm$ 8% reduction. At 51% RH, Egt+ sibs had a LE = 71 $\pm$ 1 days whereas Egt- sibs had a highly significantly lower LE = 58 $\pm$ 1 days, an 18 $\pm$ 3% reduction. We tested the hypothesis that EGT protects against uv-induced kill or mutagenesis. There were no significant differences between the germinability of Egt+ and Egt- sibs after exposure to 0 to 400 Joules/m^2 of 254 nm light. There also were no significant differences between the Egt+ and Egt- sibs in the *mtr* mutation rate to fluorophenylalanine resistance after exposure of conidia to 0 to 400 Joules/m^2 of 254 nm light. Consequently, our *in vivo* analysis indicates that EGT does not protect against uv-induced kill or mutagenesis.

44. Thiolutin inhibits protein turnover in Neurospora and yeast. Linda Lauinger, Michael Brunner, Axel Diernfellner. BZH, Heidelberg, Germany.

Proteasome inhibitors are a powerful tool for the characterization of proteins in vivo. In yeast as well as in filamentous fungi, however, the available proteasome inhibitors, like e.g. MG132 do not function due to the barrier posed by the cell wall of the organisms and an efficient evacuation of the molecules out of the cells. The dithiole thiolutin has been shown to be a potent inhibitor of RNA polymerases in prokaryotes and fungi. In the filamentous fungus Neurospora crassa, thiolutin efficiently suppresses transcription, indicating that the drug is cell permeable and not subject to a significant efflux by the multidrug resistance system. Our data indicate that thiolutin also significantly inhibits protein turnover. Concomitant with the increase in protein stability after treatment with thiolutin, we observe an accumulation of ubiquitinated protein species. Thus, our findings suggest that thiolutin may be a pleiotropic inhibitor suppressing both, RNA polymerase as well as the proteasomal activity.

45. Characterization of a Phanerochaete chrysosporium glutathione transferase reveals a novel structural and functional class with ligandin properties for wood extractive molecules. Yann Mathieu[1,2,6], Pascalita Prosper[3,4], Marc Buée[2], Stéphane Dumarçay[5], Frédérique Favier[3,4], Eric Gelhaye[1,2], Philippe Gérardin[5], Luc Harvengt[6], Jean-Pierre Jacquot[1,2], Tiphaine Lamant[1,2], Edgar Meux[1,2], Sandrine Mathiot[3,4], Claude Didierjean[3,4], Melanie Morel[1,2]. 1) Université de Lorraine, IAM, UMR 1136, IFR 110 EFABA, Vandoeuvre-les-Nancy, F-54506, France; 2) INRA, IAM, UMR 1136, Vandoeuvre-les-Nancy, F-54506, France; 3) Université de Lorraine, CRM2, UMR 7036, Vandoeuvre-les-Nancy, F-54506, France; 4) CNRS, CRM2, UMR 7036, Vandoeuvre-les-Nancy, F-54506, France; 5) Université de Lorraine, LERMAB, EA 1093, Vandoeuvre-les-Nancy, F-54506, France; 6) Laboratoire de biotechnologie, Pole Biotechnologie et Sylviculture Avancée, FCBA, Campus Foret-Bois de Pierroton, 33610 Cestas, France.

Glutathione transferases (GSTs) form a superfamily of multifunctional proteins with essential roles in cellular detoxification processes. A new fungal specific class of GST has been highlighted by genomic approaches. The biochemical and structural characterization of one isoform of this class in Phanerochaete chrysosporium revealed original properties. The three-dimensional structure showed a new dimerization mode and specific features by comparison with the canonical GST structure. An additional b-hairpin motif in the N-terminal domain prevents the formation of the regular GST dimer and acts as a lid, which closes upon glutathione binding. Moreover, this isoform is the first described GST that contains all secondary structural elements, including helix a4' in the C-terminal domain, of the presumed common ancestor of cytosolic GSTs, i.e. glutaredoxin 2. A sulfate binding site has been

identified close to the glutathione binding site and allows the binding of 8-anilino-1-naphtalene sulfonic acid (ANS). Competition experiments between ANS, which has fluorescent properties, and various molecules, showed that this GST binds glutathionylated and sulfated compounds, but also wood extractive molecules such as vanillin, chloro nitrobenzoic acid, hydroxyacetophenone, catechins and aldehydes in the glutathione pocket. This enzyme could thus function as a classical GST through the addition of glutathione mainly to phenethyl isothiocyanate but alternatively and in a competitive way, it could also act as a ligandin of wood extractive compounds. These new structural and functional properties, lead us to propose that this GST belongs to a new class that we name GSTFuA for Fungal specific GST class A.

46. Secretome analysis of *Trichoderma harzianum* cultivated in the presence of *Fusarium solani* cell wall or glucose. Marcelo HS Ramada[1,3], Andrei S Steindorff[1], Carlos Bloch Jr.[3], Cirano J Ulhoa[2]. 1) Brasilia University, Cell Biology Department, Brasilia, DF, Brazil; 2) Federal University of Goias, Biochemistry Department, Goiania, GO, Brazil; 3) EMBRAPA CENARGEN, Mass Spectrometry Laboratory, Brasilia, DF, Brazil.

Trichoderma harzianum is a fungus well known for its potential as a biocontrol agent of many fungal phytopathogens. The aim of this study was to evaluate the potential of *T. harzianum* ALL42 to control *Fusarium solani*, a phytopathogen fungus that causes several losses in common bean and soy crops in Brazil and to evaluate the secreted proteins of *T. harzianum* ALL42 when its spores were inoculated and incubated in culture media supplemented (TLE) or not (MM) with nitrogen sources and in the presence or not of *F. solani* cell walls (FsCW). In the absence of FsCW, the media were supplemented with glucose (GLU). *T. harzianum* was able to control the phytopathogen growth and started to sporulate in its area after 7 days in a dual culture assay, indicating that it had successfully parasitized the host. *T. harzianum* was able to grow in TLE+FsCW, MM+FsCW, TLE+GLU, but unable to grow in MM+GLU. Protein quantification showed that TLE+FsCW and MM+FsCW had 45 and 30 fold, respectively, more proteins than TLE+GLU, and this difference was observed in the bidimensional gels, as the two supernatants from media supplemented with FsCW had around 200 spots and the one supplemented with glucose only had 18. TLE+FsCW and MM+FsCW had above 80% of spot similarity. A total of 100 proteins were excised from all three conditions and submitted to mass spectrometry analysis. 85 out of 100 proteins were identified. The only protein observed in all three conditions is a small protein, called epl1, involved in eliciting plant-response against phytopathogens. An aspartic protease, previously described as related to mycoparasitism, was only found when *T. harzianum* was grown with glucose. Gene expression was evaluated and confirmed the gel results. In the media supplemented with FsCW, different hydrolases like chitinases, beta-1,3-glucanases, glucoamylases, alpha-1,3-glucanases, and proteases were identified. Some proteins like a small cystein-rich, alpha-L-arabinofuranosidase and NPP1, with no known function in mycoparasitism were also identified. *T. harzianum* ALL42 is able to inhibit the growth and parasitize *F. solani* and showed a complex and diverse arsenal of proteins that are secreted in response to the presence of the cell walls, with novel proteins not previously described in mycoparasitism studies.

47. Analysis of carbon catabolite repression (CCR) during cellulase formation by *Trichoderma reesei (Hypocrea jecorina)* using two-dimensional differential gel electrophoresis (2D-DIGE). Wellington Pedersoli, Lilian Castro, Amanda Antonieto, Vitor M. Faça, Roberto N. Silva. Biochemistry and Immunology, University of São Paulo, Ribeirao Preto, Sao Paulo, Brazil.

The production of cellulases by *Trichoderma reesei* (*Hypocrea jecorina*) is fundamental for the production of second generation biofuels from cellulosic wastes. The complex of cellulases of fungi *T. reesei* is strongly induced by sophorose and cellulose and also antagonized by glucose. Thus, the objective of this study was to compare the differential secretome using 2D-DIGE of *T. reesei* at induction and repression conditions. The fungus *T. reesei* (QM9414) were grown in media containing 1% cellulose for 24, 48 and 72 hours and 1% glucose for 24 and 48 hours in a orbital shaker at 180 rpm at 28°C. After filtration of each medium the supernatant was used for protein determination. Samples from each time in the different conditions were mixed in proportional amounts and submitted to isoelectric focusing in 18 cm strip of pH 4.0 - 7.0 and subsequently subjected to electrophoresis on two-dimensional gel - differential (2D-DIGE) for the separation of proteins. The comparative analysis PDQuest software (BioRad) 130 selected spots differentially expressed, wherein the inhibitor glucose showed 41 distinct protein spots and inductor cellulose 89 spots. However, only the 34 spots and 63 spots from glucose and cellulose respectively could be spotted. Identification of these spots was carried out using the mass spectrometer XEVO TQ-S (Waters). A total of 57 proteins were identified, 30 and 27 proteins from cellulose and glucose conditions respectively. The proteins identified from glucose condition were amidases, proteases, isoamyl alcohol oxidases and protein belonging to Glycosyl Hydrolases 16, 17, 43 and 54. On the other hand, the proteins identified from cellulose condition were Glycosyl Hydrolases 3, 7, 54, 55, 72, two Cip 1 (envolved in the degradation of cellulose, but with no function described), two proteases and a protein responsible for the regulation of stress. Two other proteins, one of each condition were identified as unknown. Thus, the identification of these proteins will allow a better understanding of the mechanism formation of cellulases by *T reesei* and thus contribute to improve the production of second generation ethanol. Supported by: FAPESP, CAPES and FAEPA-HC/USP-RP.

48. Transposon-associated evolution of a fungal NRPS. Daniel Berry[1], Carolyn Young[2], Paul Dijkwel[1], Barry Scott[1]. 1) Massey University, Palmerston North, New Zealand; 2) The Samuel Roberts Nobel Foundation, Ardmore, Oklahoma.

Epichloë endophytes systemically colonise the aerial tissues of cool season grasses to form mutually beneficial symbiotic associations. A defining feature of these associations is fungal synthesis of a range of secondary metabolites that protect the host from biotic and abiotic stress. One key bioprotective metabolite is the insect feeding deterrent, peramine, which is synthesized by the two-module non-ribosomal peptide synthetase (NRPS) PerA. This NRPS has an A-T-C-A-M-T-R domain structure where the Adenylation-domains provide specificity for and activation of the two amino acid substrates, the Thiolation-domains bind the reaction intermediates, the Condensation-domain catalyzes formation of the peptide bond, the Methylation-domain methylates the substrate amino acid, and the Reductase-domain cyclizes and releases the finished product from the NRPS. The *perA* gene is found exclusively within the *Epichloë*, where it is widespread among the different species, but peramine production is somewhat more discontinuous. We show that transposon-mediated deletion of the R-domain present in some *E. typhina* and *E. festucae* peramine deficient isolates is associated with a change in the predicted substrate specificity code of the first A-domain. Phylogenetic analysis of this domain groups the peramine negative isolates together whereas the phylogeny based on the whole gene matches the species phylogeny. The recombination mechanism responsible for the evolution of this novel NRPS is still to be resolved.

49. Velvet family control of penicillin production in *Penicillium chrysogenum*: PcVelB binding to isopenicillin N synthase suggests a novel regulatory mechanism. Sandra Bloemendal, Katarina Kopke, Birgit Hoff, Sarah Milbredt, Alexandra Katschorowski, Ulrich Kück. Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-University Bochum, Universitätsstr. 150, 44780 Bochum, Germany.

The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. It is supposed that subunits of the velvet complex, which were recently detected for *P. chrysogenum*, function as such global regulators, although the exact regulatory mechanism still have to be elucidated. Core components of this complex are PcVelA and PcLaeA, which regulate secondary

metabolite production, hyphal morphology, conidiation, and pellet formation [1]. Here we describe the characterization of PcVelB, PcVelC, and PcVosA as novel subunits of this velvet complex. Using yeast two-hybrid analysis and bimolecular fluorescence complementation (BiFC), we demonstrate that all velvet proteins are part of an interaction network. Functional analyses using single and double knockout strains generated by the FLP/*FRT* recombination system [2] clearly indicate that velvet subunits have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation. Most strikingly, a direct interaction of PcVelB with an enzyme of the penicillin biosynthesis pathway, the isopenicillin N synthase was identified during yeast two-hybrid analysis with PcVelB as bait. This surprising interaction was confirmed with BiFC *in vivo*, thereby localizing the interaction in dot-like structures in the cytoplasm. Our discovery of a direct interaction of the isopenicillin N synthase with a subunit of the velvet complex implies a novel regulatory mechanism how enzymes of penicillin biosynthesis are regulated at the molecular level. The results provided here contribute to our fundamental understanding of the function of velvet subunits as part of a regulatory network mediating signals responsible for morphology and secondary metabolism, and will be instrumental in generating mutants with newly derived properties that are relevant to strain improvement programs.

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[2] Kopke K, Hoff B, Kück U (2010) Appl Environ Microbiol 76:4664-4674.

50. Genome mining reveals the evolutionary origin and biosynthetic potential of basidiomycete polyketide synthases. Gerald Lackner, Mathias Misiek, Jana Braesel, Dirk Hoffmeister. Pharmaceutical Biology, Friedrich-Schiller-University Jena, Germany.

Polyketide biosynthesis is a rich source of pharmaceutically active secondary metabolites present in fungi. Besides lipid-lowering drug lovastatin, many infamous toxins are produced via this pathway. While abundant in Aspergillus, only few polyketides have been isolated from basidiomycetes. High throughput genome sequencing projects, however, now help estimate the genetic capacity of basidiomycetes to biosynthesize polyketide derivatives. By inspection of 35 sequenced basidiomycete genomes we identified and annotated 111 iterative type I and three type III polyketide synthase (PKS) genes. Phylogenetic analyses of KS genes imply that all main families of fungal PKS had already evolved before the Ascomycota and Basidiomycota diverged. A comparison of genomic data and metabolomic records shows that the number of polyketide genes surpasses the number of known polyketides considerably. This work might serve as a guide for upcoming genomic mining projects to discover novel polyketide derivates from mushrooms.

51. Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms. Hong Luo, John S. Scott Craig, Robert M. Sgambelluri, Sung-Yong Hong, Jonathan D. Walton. Department of Energy Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824, United States.

Ninety percent of fatal mushroom poisonings are caused by alpha-amanitin and related bicyclic peptides found in some species of Amanita, Galerina, Lepiota, and Conocybe. We showed that the amatoxins (mainly amanitins) and related phallotoxins are synthesized on ribosomes in A. bisporigera and the unrelated mushroom G. marginata. The primary gene products are short (34-35 amino acid) proproteins that are initially processed by a dedicated prolyl oligopeptidase. A genome survey sequence of A. bisporigera suggested that it has a repertoire of over 40 cyclic peptides, all produced on a single biosynthetic scaffold. Members of this extended gene family are characterized by conserved upstream and downstream amino acid sequences, including two invariant proline residues, flanking a six to ten-amino acid “hypervariable” region that encodes the amino acids found in the mature toxins (or predicted toxins). The evidence indicates that A. bisporigera has evolved a combinatorial strategy that could in principle biosynthesize billions of small cyclic peptides. In order to study the other steps in amanitin biosynthesis, and to engineer novel cyclic peptides, we have developed a transformation strategy for the amanitin-producing mushroom G. marginata. This first transformation method uses Agrobacterium-mediated transformation followed by hygromycin selection. Taking advantage of this platform, we are introducing artificial toxin genes that are deliberately designed to provide insights into the pathway. The synthetic genes include those that encode the cyclic octapeptide beta-amanitin, the heptapeptides phalloidin and phallacidin, examples of the toxin gene family known from A. bisporigera but not G. marginata, and randomly generated artificial sequences. Currently, thousands of transformants have been generated through an efficient pipeline and the transformants are being analyzed for production of the expected products. If successful, the novel peptides will be screened in a number of assays including RNA polymerase (the site of action of alpha-amanitin), membrane ion channels, pathogenic bacteria, and cancer cell lines.

52. Spatial assessment of oxidative and enzymatic reactions in brown rotted wood. Jon R. Menke[1], Jae San Ryu[2,5,6], Gerald N. Presley[1], Shona M. Duncan[1], Joel A. Jurgens[3], Robert A. Blanchette[3], Timothy R. Filley[4], Kenneth E. Hammel[2,5], Jonathan S. Schilling[1]. 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN; 2) Department of Bacteriology, University of Wisconsin, Madison, WI; 3) Department of Plant Pathology, University of Minnesota, St. Paul, MN; 4) Department of Earth and Atmospheric Sciences and the Purdue Climate Change Research Center, Purdue University, West Lafayette, IN; 5) Institute for Microbial and Biochemical Technology, U.S. Forest Products Laboratory, Madison, WI; 6) Eco-Friendliness Research Department, Gyeongsangnam-do Agricultural Research and Extension Services, Republic of Korea.

Brown rot fungi are theorized to use coordinated free radical oxidations and enzymatic reactions to consume wood. Though likely incompatible *in vitro*, it is proposed these reactions occur concurrently during brown rot of wood. We mapped and then compared fungal growth, wood modifications related to non-enzymatic mechanisms, and cellulase activity in thin spruce ‘wafers’ to investigate the degree of spatial coincidence of these reactions in wood degraded by *Postia placenta*. Nearly coincident oxidative and enzymatic reaction fronts were observed behind the most advanced hyphal tips, suggesting a fine-scale (likely sub-micron) spatial or biochemical extracellular mechanism may protect both hyphae and enzymes from oxidative stress. To further investigate a possible role of enzymatic reactions in the primary depolymerization of lignocellulose during brown rot, we have initiated a study to temporally assess the depth of penetration of an endoglucanase into wood cells during this process. Previous studies have used the marker proteins insulin (5.7 kDa), myoglobin (17.6 kDa), and ovalbumin (44.4 kDa); and immunocytochemical electron microscopy to demonstrate the ability of the two smaller proteins to infiltrate the cell walls of rotted wood. The *Postia placenta* endoglucanase Cel5B (PpCel5B) has a theoretical molecular weight of 34.6 kDa, which is considerably lower than the molecular weight of ovalbumin. Our approach involves using a polyclonal antibody raised against PpCel5B heterologously expressed in *Pichia pastorius*. This antibody will be used to assess the extent to which a native brown rot endoglucanase is able to penetrate *Pinus resinosa* cells. Given the common supposition that pore size prevents brown rot fungal endoglucanases from accessing wood secondary walls, even in late decay stages, this work will provide a direct assessment of enzyme ingress.

53. Molecular biological basis for statin resistance in naturally statin-producing organisms. Ana Rems, Rasmus Frandsen. DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark.

Secondary metabolites can be toxic to the organism producing them; therefore gene clusters for biosynthesis of secondary metabolites often include genes responsible for the organism’s self-resistance to the toxic compounds. One such gene cluster is the compactin (ML-236B) cluster in *Penicillium solitum*. Compactin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, and is used as a precursor for production of the cholesterol-lowering drug pravastatin. The compactin gene cluster includes two genes encoding proteins that may confer the self-resistance to compactin and its

secretion [1]. The *mlcD* gene encodes a putative 'HMG-CoA reductase-like protein', and *mlcE* encodes a putative efflux pump. However, the function of these two putative proteins has not yet been confirmed. We aim to elucidate the biological basis for compactin resistance in the compactin-producing organism. A codon-optimized version of the *mlcD* gene was inserted into the *Saccharomyces cerevisiae* genome. The constructed yeast strain was tested for sensitivity to lovastatin, a statin structurally similar to compactin, by growing the strain on media containing lovastatin. The strain showed an increased resistance to lovastatin compared to the wild-type strain. Furthermore, we investigated if MlcD confers the resistance by functional complementation of the endogenous HMG-CoA reductases in *S. cerevisiae*. There are two isozymes of HMG-CoA reductase in yeast, *HMG1* and *HMG2*, both involved in the sterol biosynthetic pathway, which leads to the synthesis of ergosterol. Following deletion of *HMG1* and *HMG2* genes in *S. cerevisiae*, we inserted the *mlcD* gene into the knockout mutants, and tested the resulted strains for sensitivity to lovastatin. The *HMG1* and *HMG2* knockout mutants were unable to grow on minimal media and had an increased sensitivity to lovastatin on rich media. However, insertion of the *mlcD* gene restored the growth of the yeast mutants and increased their resistance to lovastatin. These results show that MlcD complements the activity of the deleted HMG-CoA reductases, enabling synthesis of ergosterol in yeast. In addition MlcD confers statin resistance by being insensitive to the inhibiting effects of statins. Reference: [1] Abe Y., Suzuki T., Ono C., Iwamoto K., Hosobuchi M., Yoshikawa H. Mol Genet Genomics 2002, 267, 5:636-46.

54. Molecular genetic characterization of secondary metabolism pathways in Asperillus species. Clay Wang[1], Yiming Chiang[1], Nancy Keller[3], Kenneth Bruno[4], Scott Baker[4], Chun jun Guo[1], James Sanchez[1], Benjamin Knox[4], Alexandra Soukup[3], Jin Woo Bok[3], Manmeet Ahuja[2], Ruth Entwistle[2], Liz Oakley[2], Shu-lin Chang[1], Hsu-Hua Yeh[1], Mike Praseuth[1], Berl Oakley[2]. 1) Pharma Sci & Chemistry, Univ Southern California, Los Angeles, CA; 2) Department of Molecular Biosciences, University of Kansas; 3) Department of Medical Microbiology and Immunology and Department of Bacteriology, University of Wisconsin Madison; 4) Pacific Northwest National Laboratory.

Advances in next generation DNA sequencing have provided a large number of fungal genome sequences in public databases. Within these genomes are large numbers of cryptic secondary metabolism pathways. Data will be presented where we use a comparative genomics approaches to identify the products of these cryptic pathways. Next we use a gene knock out approach to create mutants followed by isolation and characterization of intermediates and shunt products. Using this approach we have been able to identify the products of a meroterpenoid pathway in A. terreus.

55. A branched biosynthetic pathway is involved in production of roquefortine and related compounds in Penicillium chrysogenum. Hazrat Ali[1,2], Marco Ries[3], Jeroen Nijland[1,2], Peter Lankhorst[4], Thomas Hankemeier[3,5], Roel Bovenberg[4,6], Rob Vreeken[3,5], Arnold Driessen[1,2*]. 1) Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, Zernike Institute for Advanced Materials, University of Groningen, The Netherlands; 2) Kluyver Centre for Genomics of Industrial Fermentations, Julianalaan 67, 2628BC Delft, The Netherlands; 3) 3Division of Analytical Biosciences, Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands; 4) DSM Biotechnology Center, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands; 5) Netherlands Metabolomics Centre, Leiden University, Leiden, The Netherlands; 6) Synthetic Biology and Cell Engineering, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands.

Profiling and structural elucidation of secondary metabolites produced by the filamentous fungus Penicillium chrysogenum and derived deletion strains were used to identify the various metabolites and enzymatic steps belonging to the roquefortine/meleagrin pathway. Major abundant metabolites of this pathway were identified as histidyltryptophanyldiketopiperazine (HTD), dehydrohistidyltryptophanyldiketopiperazine (DHTD), roquefortine D, roquefortine C, glandicoline A, glandicoline B and meleagrin. Specific genes could be assigned to each enzymatic reaction step. The nonribosomal peptide synthetase RoqA accepts histidine and tryptophan as substrates leading to the production of the diketopiperazine HTD. DHTD, previously suggested to be a degradation product of roquefortine C, was found to be derived from HTD involving the cytochrome P450 oxidoreductase RoqR. The dimethylallyltryptophan synthetase RoqD prenylates both HTD and DHTD yielding the products roquefortine D and roquefortine C, respectively. This leads to a branch in the otherwise linear pathway. Roquefortine C is subsequently converted into meleagrin with glandicoline A and B as intermediates, involving two monooxygenases (RoqM and RoqO) and a methyltransferase (RoqN). It is concluded that roquefortine C and meleagrin are derived from a branched biosynthetic pathway rather than a linear pathway as suggested in literature.

56. A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, *Leptosphaeria maculans*. Candace Elliott[1], Damien Callahan[2], Daniel Schwenk[3], Markus Nett[4], Dirk Hoffmeister[3], Barbara Howlett[1]. 1) School of Botany, University of Melbourne, Melbourne, Australia; 2) Metabolomics Australia, School of Botany, The University of Melbourne, Victoria 3010, Australia; 3) Friedrich-Schiller-Universität, Department Pharmaceutical Biology at the Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany; 4) Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany.

Phomenoic acid, a long chain aliphatic carboxylic acid, is a major metabolite produced by *Leptosphaeria maculans*, a fungal pathogen of *Brassica napus* (canola). Early biosynthetic studies suggested that the methyl group derived from S-adenosylmethionine (SAM), whereas the incorporation pattern of [13C] acetate suggested a polyketidic origin of the linear portion of phomenoic acid (Devys et al., 1984). We have used domain modelling to predict a candidate polyketide synthase (PKS) for phomenoic acid biosynthesis. Of the 15 predicted polyketide synthases (PKS) in the *L. maculans* genome, seven were reducing with the appropriate domains (KS - keto-synthase; AT - acyltransferase; DH - dehydratase; MT- methyltransferase; ER - enoylreductase; KR - ketoreductase; ACP- acyl carrier protein) for the biosynthesis of phomenoic acid. The most highly expressed of these seven genes, *PKS2*, was silenced to 10% of that of wild type levels and the resultant mutant produced 25 times less phomenoic acid than the wild type did, indicating that *PKS2* is involved in phomenoic acid biosynthesis. This gene is part of a cluster and nearby genes are co-regulated. A two-fold reduction in the expression of the adjacent transcriptional regulator *C6TF*, led to at least a 20-fold reduction in expression of *PKS2*, as well as of other genes in the cluster (*P450*, *YogA*, *RTA1* and *MFS*), but not of the adjacent *ChoK* or a hypothetical gene (*Hyp*). This down-regulated mutant also showed a marked reduction in phomenoic acid production. Phomenoic acid is toxic towards another canola pathogen *Leptosphaeria biglobosa* 'canadensis', but *L. maculans* and to a lesser extent the wheat pathogen, *Stagonospora nodorum* are more tolerant. Phomenoic acid may play a role in allowing *L. maculans* to outcompete other fungi in its environmental niche.

57. Exploring and Manipulating Pleuromutilin Production. Patrick M Hayes[1], Russell J Cox[2], Andy M Bailey[1], Gary D Foster[1]. 1) School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK; 2) School of Chemistry, University of Bristol, Bristol BS8 1TS, UK.

Antibiotic resistance has arisen in a significant number of human pathogens, antibiotic discovery and development is, however, currently not keeping pace. This has lead to the reinvestigation of some naturally produced antibiotic compounds which act in a manner that avoids common resistance mechanisms. Pleuromutilin is one such compound with activity against bacteria such as Methicillin Resistant Staphylococcus aureus (MRSA). Pleuromutilin is generally synthesised at a low titre by its native producer Clitopilus passeckerianus and as such research into its biosynthesis may enable yield increases. This project has taken a multifaceted approach to manipulate the Pleuromutilin biosynthetic gene cluster in a variety of fungal organisms. Within C.

passeckerianus gene silencing is being used to interfere with specific stages of biosynthesis, silenced transformed lines have been analysed both chemically, to determine the impact of silencing on the metabolome of the organism, and via QRT-PCR, to determine the impact of silencing on the transcription of the gene cluster. Heterologous expression of the entire gene cluster is being evaluated and analysed in the model basidiomycete Coprinopsis cinerea. Engineered expression of the biosynthetic genes is being performed in the industrially relevant ascomycete Aspergillus oryzae to build the pathway in a stepwise manner. Progress in each of these areas will be presented.

58. Improvement of *Monascus pilosus* for the production of functional foods by overexpression of the *laeA* gene. In H. Lee, Sang S. Lee, Jin H. Lee. Dept. of Advanced Fermentation Fusion Science & Technology, Kookmin Univ, Seoul, South Korea.

Filamentous fungi *Monascus* species have been used to ferment rice producing red mold rice (RMR). They produce several bioactive compounds during fermentation, however, they should have a potential to produce other bioactive compounds considering that most of fungi has many silent secondary metabolite (SM) gene clusters. Therefore, we thought that *Monascus* species could be improved for functional food production by activation of such silent SM gene clusters. We overexpressed the *laeA* gene that is known to encode a global positive regulator of secondary metabolism under the *alcA* promoter in *Monascus pilosus*. An *OE::laeA* transformant produced more secondary metabolites including ones not detected under an uninduced condition. RMR fermented with the *Monascus pilosus OE::laeA* contained 7 times more monacolin K, a cholesterol lowering agent, than non-transformants increasing from 2.45 to 15.59 mg/kg. In addition, the production of pigments was remarkably increased and antioxidant activity was increased as well. This study suggests that *Monascus* species that are important industrial fermentative fungi in Asia could be improved for the production of functional foods by overexpression of the *laeA* gene.

59. Molecular genetics studies on secondary metabolism in *Chaetomium globosum* reveal involvement of aureonitol and chaetoglobosins in gene regulation and sexual reproduction. Takehito Nakazawa, kan'ichiro Ishiuchi, Satoru Sugimoto, Yasutaka Gotanda, Michio Sato, Hiroshi Noguchi, Kenji Watanabe. Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan.

Chaetomium globosum has been reported to produce many natural products, secondary metabolites, with complex structures biosynthetic pathways of which are very interesting to be elucidated such as aureonitol, chaetoglobosins, chaetocins and chaetoviridins. Recently, we developed molecular genetics systems for understanding the secondary metabolism in this fungus: High frequency gene targeting by *Cg.ligD* disruption and the *pyrG* marker recycling. Then, we identified biosynthetic gene clusters for various natural products that had been isolated from *C. globosum*, and analyzed their biosynthetic mechanisms/pathways. We also obtained new natural products by changing an epigenetic regulation. During these studies, we found that some of mutations in biosynthetic genes allowed us to obtain interesting phenotypes: drastic changes in secondary metabolism profiles and defects in production of sexual spores. Here, we present that aureonitol and chaetoglobosins would play a critical role of controlling the productivity of secondary metabolites and producting of sexual spores, respectively. A mutated gene of *artH* responsible for biosynthesizing aureonitol exhibits activating chaetoviridins biosynthesis and inactivating chaetoglocin A biosynthesis. A qRT-PCR analysis shows that transcriptional expressions of biosynthetic genes for chaetoviridins are activated, whereas those for chaetoglocins are inactivated by the mutated *artH* gene. Supplementation with aureonitol toDartH strain is observed to inactivate chaetoviridins biosynthesis and transcriptional expression of their biosynthetic gene cluster. These results strongly suggest that aureonitol could be involved in transcriptional regulation of secondary metabolism in *C. globosum*. On the other hand, we also find chaetoglobosins is essential for production of sexual spores: Mutations in its biosynthetic genes clearly impair meiotic process. It is anticipated that chaetoglobosins are involved in meiotic process, because the mutants don't affect formation of fruiting bodies (perithecia). Previously, chaetoglobosins were shown to inhibit actin polymerization in vitro. Therefore, chaetoglobosins would play a role of regulating actin or actin-related protein in *C. globosum*, which has been reported to be associated with regulation of meiotic process as well as morphogenesis in fungi.

60. Identification of T. asperellum CAZyme genes. Lasse Bech[1], Morten Nedergaard Grell[1], Peter Kamp Busk[1], Hai Zhao[2], Lene Lange[1]. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, PR China.

To understand the complexity of enzymatic hydrolysis of plant cell walls and to discover new enzymes, one approach is to analyze cell wall degrading enzymes (CWDEs) in the transcriptome of plant degrading fungi like species of the genus Trichoderma. The Trichoderma spp. are ubiquitous colonizers of cellulosic materials and are often be found where decaying plant material is available as well as in the rhizosphere of plants. Trichoderma spp. are successful colonizers of their habitats, which is shown both by their efficient utilization of the biomass as well as their secretion capacity for antibiotic metabolites and enzymes. This study shows the transcriptome of Trichoderma asperellum isolated from duckweed. Duckweed is an aquatic plant that has been shown to clean eutrophic water reservoirs for the production of biomass, which can be used as feed, fertilizer and fuel through the biorefinery platform. The fungus was optimized for the production of CWDEs, where more than 200 glucoside hydrolases from 47 different families were identified. Several group members exhibited novel traits such as larger residue differences in active site and substrate binding site, hence making them interesting subject for expression and further characterization. This data was compared with the hydrolysis of duckweed by enzyme blend from T. asperellum. The enzyme blend showed a promising degree of hydrolysis of duckweed indicating that T. asperellum is a candidate for on-site enzyme production for the enzymatic hydrolysis of certain duckweed species. The transcriptome data can further be used to map the expression of CWDEs under different conditions thereby coming closer to understanding the relationship between CWDEs and the plant cell wall.

61. Identification of a lactose permease of Trichoderma reesei that is required for cellulase gene expression. Christa Ivanova[1], Jenny Bååth[2], Bernhard Seiboth[1,3], Christian Kubicek[1,3]. 1) Institute of Chemical Engineering, University of Technology of Vienna, 1060 Vienna, Austria; 2) Lund University, SE-221 00 Lund, Sweden; 3) Austrian Institute of Industrial Biotechnology (ACIB) GmBH c/o Institute of Chemical Engineering, University of Technology of Vienna, 1060 Vienna, Austria.

The disaccharide lactose has been shown to be a potent inducer of cellulases in T. reesei, and it is virtually the only soluble waste product that can be used for commercial enzyme production. To understand the complex regulatory mechanisms underlying cellulase induction by lactose, we performed comparative transcriptomic analysis using oligonuclotide arrays. Among the 410 genes that were upregulated over four-fold on lactose, were all cellulases, cellulase-enhancing proteins, major hemicellulases and also 63 MFS- (major facilitator superfamily) -permeases. The MFS permeases are characterized by 12-transmembrane helices and a well conserved motif between TMS (transmembrane segment) 2 and 9. In order to investigate the function of these transporters, we generated deletion strains in T. reesei. For this, the 14 most-upregulated transporter encoding genes were chosen. One of these disruptant strains showed strongly impaired growth on lactose and was therefore chosen for further analysis. The strain showed impaired growth on lactose, whereas growth on glucose, glycerol and cellobiose remained unaltered, suggesting that the transporter is required for lactose uptake. The strain was devoid of cellulase gene expression during cultivation on lactose, whereas it formed cellulases upon incubation with sophorose suggesting that it is

involved in cellulase induction by this disaccharide. The deletion of the other 13 transporter encoding genes led to no reduction of growth on any carbohydrate (including glucose and cellobiose), probably due to redundancy of their function. Our data show that lactose leads not only to the formation of a complete cellulase and hemicellulase system in T. reesei, but also to the transcription of a plethora of transporters likely associated with the uptake of the hydrolysis products.

62. The diversity of the Mannosylerythritol lipids depends on the peroxisomal targeting of the Mannosylerythritol acyl transferases Mac1 and Mac2 in *Ustilago maydis*. Johannes Freitag[1], Julia Ast[1], Uwe Linne[2], Elisa Leisge[1], Michael Bölker[1], Björn Sandrock[1]. 1) Biology - Genetics, Philipps-University Marburg, Marburg, Germany; 2) Chemistry, Philipps-University Marburg, Marburg, Germany.

Under nitrogen starvation the smut fungus *Ustilago maydis* produces a bunch of secondary metabolites. Among these are the glycolipids Ustilagic acid (UA) and Mannosylerythritol lipid (MEL), which consist of a sugar moiety esterified with fatty acid side chains of variable length (from C2 - C18). The biosynthesis of UA is encoded by the UA gene cluster (11 genes). MEL production depends on the MEL gene cluster composed of the genes *mat1*, *mmf1*, *mac1*, *emt1* and *mac2*. Deletion of mac1, mac2 or emt1 in U. maydis resulted in the complete loss of MELs. Medium-length fatty acids (C4-C14) are derived from longer fatty acids (C16-C18) by partial peroxisomal b-oxidation. After bioinformatic analysis we have identified bona fide peroxisomal targeting sequences 1 (PTS1) at the C-termini of the two Mannosylerythritol lipid acyltransferases Mac1 and Mac2 but not in any other protein involved in the biosynthesis of the MELs or the UAs. Here we show that Mac1 and Mac2 localize in peroxisomes, and that this localization depends on the PTS1 motifs. The analysis of glycolipid production by thin layer chromatography and mass spectrometry from wild type strain MB215 revealed a mixture of MELs with different length of the fatty acid side chains ranging from C12, C14 and C16. Strains expressing both cytoplasmic variants Mac1DPTS and Mac2DPTS showed a reduction of diversity of MELs. In these mutants MELs with C16 and C2 side chains are significantly overrepresented. This indicates that MEL production is coupled to peroxisomal b-oxidation resulting in a more variable distribution in the length of fatty acid side chains. Currently, we investigate the MEL production is strains lacking peroxisomes and the importance of MEL diversity for the life of *U. maydis*.

63. Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers. Carol E. Davis, Howard S. Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521.

Phytophthora infestans is the causative agent of late blight and was responsible for the Irish famine in the 1840's. Today it still continues to be a global problem and in the USA it has been reported that the economic loss on potato crops alone exceeds $6 billion per year. A successful phytopathogenic relationship depends on the ability of the organism to adapt its metabolism during infection on various nutritional substrates (e.g., plant versus tuber) and at different times throughout infection when nutrients may be limiting. Investigation of this metabolic adaptation is key to understanding how *P. infestans* succeeds as a pathogen. To do this, tomato plants and potato tubers were infected with zoospores using a "dipping" method. RNA was extracted at 3 dpi and 6 dpi and subsequently used in library preparation. Following this, the libraries were quality checked by analysis on a Bioanalyzer using a high sensitivity DNA chip. Using Illumina technology (50 bp, paired-end reads) RNA Sequencing was performed. For each sample an average of 262 million reads was obtained. As a reference for the *in planta* data, RNASeq was also performed on defined and complex media. Mining of the data shows that the expression profiles of some pathways change, such as glycolysis and gluconeogenesis. Learning how metabolic adaptation occurs will prove useful in the development of novel control strategies for this plant pathogen.

64. Multi-copper oxidase genes of *Heterobasidion irregulare*. Ming-Chen Hsieh, Bastian Doernte, Ursula Kües. Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany.

The species complex *Heterobasidion* consists of well-known wood decomposers that infect mainly conifers. The fungi are white rots that decay lignin and cellulose. Laccases are enzymes that potentially attack the lignin. In the sequenced genome of the North American species *H. irregulare* 18 multi-copper oxidase genes (mco) are found (1). Phylogenetic sequence analysis divides the encoded proteins into five subclusters of mcos. In total, 14 proteins clustered in two different subfamilies of classical laccases whereas two others are found amongst ferroxidases/laccases (enzymes with often dual activities), one under fungal Fet3-type ferroxidases and one with fungal ascorbate oxidases. The potential three-dimensional structures of all mcos were predicted by homology modelling for further grouping. Six of the potential laccase genes were first chosen for subcloning and expression in the heterologous basidiomycete *Coprinopsis cinerea*. (1) Olson et al. (2012). Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytologist 194: 1001-1013.

65. The two novel class II hydrophobins of *Trichoderma* stimulate enzymatic hydrolysis of polyethylene terephthalate (PET). Liliana E. Tenorio-Rammer[1], Doris Ribitsch[1], Annemarie Marold[1], Katrin Greimel[1], Enrique Herrero Acero[1], Georg M. Guebitz[1,2], Christian Kubicek[1,3], Irina S. Druzhinina[1,3]. 1) ACIB - Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria; 2) c/o Institute for Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria; 3) Microbiology Group, Institute of Chemical Engineering, Vienna University of technology, Vienna, Austria.

Polyethylene terephthalate (PET), a thermoplastic polyester with excellent industrial properties, can be functionalised and/or recycled via hydrolysis by microbial cutinases. Here we tested whether hydrophobins (HFBs), small secreted fungal proteins containing eight positionally conserved cysteine residues, would be able to enhance the rate of enzymatic hydrolysis of PET. To this end, we selected the genus of the mycoparasitic filamentous fungus *Trichoderma* as it has been previously shown to have the most proliferated arsenal of HFBs among all fungi. Consequently we used the phylogenetic approach to identify the two novel class II HFBs (HFB4 and HFB7) from *Trichoderma* as the first candidates for the test. HFB4 and HFB7, produced in *E. coli* as N-terminal glutathione-S-transferase fusion proteins, exhibited subtle structural differences reflected in the hydropathy plots which were correlated with unequal hydrophobicity and hydrophily respectively determined by water contact angle measurements. However they exhibited a dosage-dependent stimulation of PET hydrolysis by cutinase from *Humicola insolens* with HFB4 displaying an adsorption isotherm, whereas HFB7 was active only at very low concentrations and behaved inhibitory beyond them. We conclude that class II HFBs can stimulate the activity of cutinases on PET, but individual HFBs can display different properties in this process thus warranting a broader screening of HFBs for such industrial applications.

66. Analysis of polyketide synthase gene clusters in *Cladonia metacorallifera* genome. J.-S. Hur[1], J. A. Kim[1], Y. J. Koh[1], S. Kim[2]. 1) Korean Lichen Research Center, Sunchon National University, Sunchon, South Korea; 2) Wildlife Genetic Resources Center, National Institute of Biological Resources, Korea.

Lichen-forming fungi produce highly diverse and unique secondary compounds such as depsides, depsidones, dibenzofurans and depsones. The biosynthesis of secondary metabolites is governed by polyketide synthase (PKS). However, the molecular mechanisms underlying the biosynthesis of these metabolites are poorly understood. Here we present analysis of the structure of the PKS gene clusters responsible for secondary metabolite production in the recently sequenced genome of lichen-forming fungus Cladonia metacorallifera. We found 37 type I polyketide synthase genes which were composed of 19 reducing PKSs, one partial reducing PKS and 17 non-reducing PKSs. Lichen-forming fungal PKS domains shared common structure with filamentous fungal PKSs. Phylogenetic analysis shows that some lichen-forming fungal PKSs constructed an unique clade in other filamentous fungal PKS clades.

67. Inhibition of benzoate 4-monooxygenase (CYP53A15) from *Cohliobolus lunatus* by cinnamic acid derivatives. Branka Korosec[1], Barbara Podobnik[2], Sabina Berne[3], Neja Zupanec[1], Metka Novak[1], Nada Krasevec[1], Samo Turk[4], Matej Sova[4], Ljerka Lah[1], Jure Stojan[3], Stanislav Gobec[4], Radovan Komel[1,3]. 1) National Institute of Chemistry, Ljubljana, Slovenia; 2) Lek Pharmaceuticals d.d., Verovskova 57, SI-1000 Ljubljana, Slovenia; 3) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia; 4) Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Askerceva cesta 7, SI-1000 Ljubljana, Slovenia.

Fungal infections cause huge economic losses in agriculture. Some of the major phytopathogens also cause serious, and very often lethal, infections in human and animals. Plants may be a good source of antifungals since they have to defend themselves by producing numerous secondary metabolites, such as sterols, terpens, polycosanols and phenolic compounds. Successful development of antifungal compounds, based on natural defense molecules, could prove useful in combating infectious and toxin-producing fungi in both agriculture and medicine. In recent years several promising antifungal targets have been under exploration. One of such is also fungal CYP53, member of the family of highly conserved CYP proteins, involved in detoxification of benzoate, a key intermediate in metabolism of aromatic compounds in fungi. High specificity and absence of homologue in higher eukaryotes assign CYP53A15 from the filamentous fungus *Cochliobolus lunatus* as interesting drug target. In our latest research we explored chemical properties of isoeugenol for ligand-based similarity searching, and the homology model of CYP53A15 of *Cochliobolus lunatus*, for structure-based virtual screening of a composite chemical library. Two cinnamic acid derivatives were amongst the highest scoring compounds. In the past few years, several other reports about antifungal activity of cinnamic acid derivatives have been published. In order to investigate the potential inhibitory activity on benzoate 4-monooxygenase (CYP53A15) we analyzed antifungal activity of 9 commercially available, and 10 representative cinnamic acid derivatives from our library. Furthermore, to obtain more information about structure-activity relationship 26 additional cinnamic acid esters and amides were synthesized and included in our assays. Among 45 cinnamic acid derivatives tested, 7 compounds have shown antifungal activity against *C. lunatus*, *A. niger* and *P. ostreatus* in in vivo inhibition tests. Compounds with antifungal activity were further evaluated for inhibition of CYP53A15 activity with spectral binding titration assay and HPLC. The best two inhibitors of CYP53A15 activity showed 70% inhibition at 600 mM concentration and were selected for further optimization of new lead structures.

68. Higher yields of cyclodepsipetides from *Scopulariopsis brevicaulis* by random mutagenesis. Linda Paun[1], ElKbir Hihlal[1], Annemarie Kramer[2], Antje Labes[2], Johannes Imhoff[2], Frank Kempken[1]. 1) Botanical Institute, Christian-Albrechts-University, Kiel, Germany; 2) Kieler Wirkstoff-Zentrum KiWiZ at GEOMAR, Kiel, Germany.

The ascomycete *Scopulariopsis brevicaulis*, which was isolated from the marine sponge *Tethya aurantium*, produces two cyclodepsipeptides, scopularides A and B [1]. Both peptides exhibit activity against several tumor cell lines. Within the EU-project MARINE FUNGI (EU FP7, 265926) one of our aims is to enhance the production of these secondary metabolites. We are in the process to establish two ways of random mutagenesis by both UV radiation and transposon-mediated. To this end we created UV-mutants and a miniaturised screening method was developed. UV-radiation was performed at 312 nm and the survival rate was set to 1 %. With this method a mutant library was established. To screen these mutants for higher secondary metabolites production, we developed a screening method which includes decreased cultivation volume, fast extraction and an optimised LC-MS analysis format. Using the UV mutagenesis, we identified several mutants with a higher scopularide production in comparison to the wild type. One of these mutants, which produces three times more biomass and more than double the amount of scopularide A, has been used for another round of mutation. Next generation sequencing is being employed to identify the molecular genetic basis of the observed mutations. In parallel we employ transposable elements to introduce mutants [2]. The impact of transposons on gene expression as well as their ability to cause major mutations within the genome makes them an interesting tool for random mutagenesis [3, 4, 5]. We employ the *Vader* transposon in its homologous host and found that it mostly integrates within or very close to genes thus it appears to be a useful tool for transposon-mediated mutagenesis in *A. niger* (6). At current we try to enhance its usability by modifying the *Vader* element. [1] Yu, Z.; Lang, G.; Kajahn, I.; Schmaljohann, R.; Imhoff, J. J. Nat. Prod. 2008, 71, 1052-1054 [2] Braumann I, van den Berg M, & Kempken F (2007) Fungal Genet Biol 44(12):1399-1414. [3] Daboussi MJ & Capy P (2003) Annu Rev Microbiol 57:275-299. [4] Kempken F (2003) Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics, eds Arora DK & Khachatourians GG (Elsevier Science Annual Review Series), pp 83-99. [5] Kempken F & Kück U (1998) BioEssays 20:652-659. [6] Hihlal E, Braumann I, van den Berg M, Kempken F (2011) Appl Environment Microbiol, 77: 2332-2336.

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69. Generation of pathogenic diploids from heterogeneous conidial populations of Aspergillus flavus. Farhana Runa[1], Ignazio Carbone[1], Deepak Bhatnagar[2], Gary Payne[1]. 1) Plant Pathology, North Carolina State University, Raleigh , NC; 2) Southern Regional Research Center, USDA, New Orleans, LA.

Aspergillus flavus, a major producer of aflatoxin, has emerged as an opportunistic pathogen for a wide range of hosts. Understanding genetic variation within strains of A. flavus is important for controlling disease and reducing aflatoxin contamination. Because conidia of A. flavus are multinucleated but haploid, we wanted to know if nuclear condition or ploidy of conidia could be potential sources of genetic variation. The objective of our study is to detect nuclear heterogeneity and ploidy in conidial populations of A. flavus and determine their impact on fungal ecology. In order to examine heterokaryosis, protoplast of two different auxotrophic strains in which nuclei were labeled with yellow (EYFP) and cyan (ECFP) fluorescent markers were fused. Fusants between the two strains were obtained through polyethylene mediated cell fusion and selection on minimal medium, which favored the growth of the fusants over that of either parental strain. Fusants selected for further study showed heterogeneous conidial populations with nuclei predominantly expressing either EYFP or ECFP, or a very few expressing both EYFP+ECFP. Conidia containing nuclei expressing only EYFP+ECFP were separated by Fluorescence-Activated Cell Sorting (FACS) and found to contain both yellow and cyan fluorescent proteins in the same nuclei. Further characterization of conidia having only one nucleus, but expressing both EYFP+ECFP, showed them to be diploids. Pathogenicity assays using Galleria mellonella showed that

diploids are more virulent than the parental haploids. Our results suggest that conidial populations of A. flavus are predominantly homokaryotic but a small percentage of conidia are heterokaryotic. Within a heterokaryon, a diploid nucleus could be formed by fusion of two haploid nuclei, which may allow the generation of a pathogenic strain.

70. Inhibition of appressorium formation of *Magnaporthe oryzae* by roxithromycin and its possible molecular target. Akira Ishii, Mayu Kumasaka, Megumi Narukawa, Takashi Kamakura. Applied Biological Science, Tokyo Univ. of Science, Noda, Chiba, Japan.

Roxithromycin (RXM), a 14-membered macrolide which was originally active against prokaryote, has beneficial side effects such as anti-inflammatory activities were reported and actually applied to human. However, the mechanisms underlying these side effects are still unclear. In this study, we found that RXM inhibited appressorium formation of rice blast fungus *Magnaporthe oryzae* (*M. oryzae*). These results suggest that there are alternative targets in broad eukaryotic organisms and it is interesting to identify the molecular target of the secondary effect on human using *M. oryzae*. Magnaporthe oryzae is the causal agent of rice-blast disease. *M. oryzae* enters its host plant using a specialized infection structure known as an appressorium. The developmental stage of appressorium is sensitive to various chemical inhibitors, because large numbers of genes are involved in cellular differentiation. Since appressorium formation by *M. oryzae* can be observed on artificial surfaces, it can be a useful tool to search new activity of various chemicals. We performed phage display to search novel molecular target(s) of the antibiotic. We found that one candidate gene *32-11* may play important roles in appressorium formation. Expression of *32-11* gene, in a *32-11* mutant, was lower than wild type during developing infection structure, and the mutant was less affected by RXM. Although germinate and formation of appressoria were normal. Over expression of *32-11* gene caused no effect to RXM sensitivity, germination nor appressorium formation compared with the wild type. Over expression of *32-11* caused no effect to RXM activity, germination or appressorium formation compare to the wild type. To investigate whether lower expression of *32-11* causes the less sensitivity to RXM, we introduced *32-11* over expression vector into *32-11* reduced mutant. These mutants restored their wild type phenotype. These results possibly suggest that the complex of 32-11 product and RXM affects another molecule which plays an important role in appressorium formation at *M. oryzae*.

71. Identification of novel genes involved in induction of appressorium development triggered by plant-derived signals in *Colletotrichum orbiculare*. Sayo Kodama[1], Ayumu Sakaguchi[2], Yasuyuki Kubo[1]. 1) Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto Prefectural University, Kyoto, Japan; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan.

Many plant pathogenic fungi initiate infection of host leaves by the germination of conidia and differentiation of appressoria at the tip of germ tubes. These morphological changes are triggered by various external signals such as physical or chemical signals from the plant surface. In our previous study, cucumber anthracnose fungus *Colletotrichum orbiculare CoKEL2*, a *Schizosaccharomyces pombe tea1* homologue, encoding a kelch repeat protein was identified. The *cokel2* mutants formed abnormal appressoria on glass slides, and those appressoria were defective in penetration hyphae development into cellulose membranes, an artificial model substrate for fungal infection. In contrast, the *cokel2* mutants formed normal appressoria on the host cucumber plant and retained penetration ability. Moreover, when conidia were incubated in the presence of exudates from cucumber cotyledon, normal appressorium formation on the artificial substrate by the *cokel2* mutants was restored. These results suggest that *CoKEL2* is essential for normal morphogenesis of appressoria and that there is a bypass pathway that transduces plant-derived signals for appressorium formation independent of *CoKEL2*. These plant-derived signaling pathways for appressorium formation have not been characterized in fungal pathogens including *C. orbiculare*. To determine specific components of the plant-derived signaling pathway that leads to appressorium formation, we screened six *cokel2* double mutants that formed abnormal appressoria not only on artificial substrates but also on the host plant surface. Furthermore, reintroduction of *CoKEL2* into those *cokel2* double mutants restored normal appressorium formation on artificial substrates, suggesting that *cokel2* double mutants have defects in *CoKEL2*-independent and plant-derived specific signaling pathway for appressorium formation. We identified and characterized candidate mutated genes by whole genome sequencing of the six *cokel2* double mutants. To define the involvement of those candidate mutated genes in appressorium formation, we observed the phenotypes of candidate gene*D* single mutants, *cokel2D* candidate gene*D* double mutants, and complementation strains. As expected, candidate gene*D* mutants in *cokel2D* back ground showed same phenotypes as those of screened *cokel2* double mutants.

72. Unique protein domains regulate *Aspergillus fumigatus* RasA localization and signaling during invasive growth. Rachel V. Lovingood[1], Praveen R. Juvvadi[2], William J. Steinbach[2], Jarrod R. Fortwendel[1]. 1) Microbiology and Immunology, University of South Alabama, Mobile AL, USA; 2) Pediatric Infectious Diseases, Duke University, Durham NC, USA.

Invasive pulmonary aspergillosis (IPA) is propagated by inhalation of *A. fumigatus* spores that germinate and invade the lung tissue in search of nutrients. We have shown that the *A. fumigatus* RasA GTPase protein is necessary for hyphal morphogenesis, cell wall integrity, and virulence during IPA. Our previous studies focused on conserved protein domains regulating RasA localization and signaling. These studies revealed the requirement for plasma membrane (PM)-localized Ras for proper signaling and regulation of *A. fumigatus* growth and virulence. Therefore, mechanisms controlling Ras localization are of interest in designing novel antifungal Ras inhibitors. Although Ras pathways may represent valid antifungal targets, the importance of fungal-specific Ras protein domains to Ras function in fungal pathogenesis remains unexplored. To address this important knowledge gap, we identified fungal-specific Ras protein domains by comparing fungal Ras sequences to their human counterpart, H-ras. We hypothesized that such domains could serve as targetable areas to selectively inhibit the fungal Ras protein. This analysis revealed two areas of significant divergence with H-ras: i) the Invariant Arginine Domain (IRD), a novel domain conserved in the RasA homologs of every available fungal genome but not present in H-ras and ii) an extended hypervariable region (HVR). Truncation analysis of the HVR identified a serine-rich region that is necessary for localization to the PM and for RasA signaling during hyphal morphogenesis. Interestingly, mutational analysis of the IRD produced a properly localized yet non-functional RasA protein. However, activation of the IRD RasA mutant was not altered suggesting a role for the IRD during interactions of RasA with downstream effectors. Further characterization of the IRD and HVR, and the protein interactions to which they contribute, will reveal fungal-specific aspects of Ras function and may define a new paradigm for Ras signal transduction in fungal organisms.

73. Light regulates growth, stress resistance and metabolism in the fungal pathogen *Aspergillus fumigatus*. Kevin K. Fuller, Carol S. Ringleberg, Jennifer J. Loros, Jay C. Dunlap. Genetics, Geisel School of Medicine at Darmouth, Hanover, NH.

Light serves as an important environmental cue that influences developmental and metabolic pathways in a variety of fungi. Interestingly, orthologs of a conserved blue light receptor, WC-1, promote virulence in two divergently related pathogenic species, *Cryptococcus neoformans* and *Fusarium oxysporum*, suggesting that photosensory systems may be conservatively linked to fungal pathogenesis. *Aspergillus fumigatus* is the predominant mold pathogen of immunocompromised patients, but if and how the organism responds to light has not been described. In this report, we demonstrate that the fungus can indeed sense and distinctly respond to both blue and red portions of the visible spectrum. Included in the *A. fumigatus* photoresponse is a reduction in conidial germination kinetics, increased hyphal pigmentation, enhanced resistance to acute ultra-violet and oxidative stresses, and an increased

susceptibility to cell wall perturbation. Through gene deletion analysis we have found that the WC-1 ortholog, LreA, is a bone fide blue light receptor in *A. fumigatus* that is required for the photopigmentation response. However, the *DlreA* mutant retains several blue light mediated responses, including the germination and stress resistance phenotypes , suggesting other blue light receptors are operative in this fungus. We also show that the putative red light sensing phytochrome, FphA, is involved with some, but not all, blue light specific phenotypes, indicating a complex interaction between red and blue light photosystems in *A. fumigatus*. Finally, whole genome microarray analysis has revealed that *A. fumigatus* displays broad patterns of gene induction and repression upon exposure to light. Affected genes are largely metabolic and include those involved in lipid and sterol synthesis, respiration, carbohydrate catabolism, amino acid metabolism and metal ion homeostasis. Taken together, these data demonstrate the importance of the photic environment on the physiology of *A. fumigatus* and provide a foundation for future studies into an unexplored area of this important pathogen.

74. Analysis of critical domains in calcineurin A required for septal targeting and function in *Aspergillus fumigatus*. Praveen R. Juvvadi[1], Jarrod R. Fortwendel[2], Christopher Gehrke[1], Frédéric Lamoth[1], William J. Steinbach[1]. 1) Department of Pediatrics, Duke University Medical Center, Durham, NC; 2) Department of Microbiology and Immunology, University of South Alabama, Mobile, AL.

Calcineurin, a calmodulin (CaM)-dependent protein phosphatase, is known to play key roles in virulence, growth and stress responses of pathogenic fungi. Critical understanding of calcineurin regulation and identifying the residues indispensable for calcineurin activity *in vivo* will pave the way for devising new drug targets for combating invasive aspergillosis. Previous studies from our laboratory showed that the calcineurin complex (CnaA and CnaB) in *Aspergillus fumigatus* selectively localizes at the hyphal tip and septum to direct proper hyphal growth and regular septum formation. However, the domains responsible for targeting and function of CnaA at the hyphal septum remain unknown. Here we performed extensive truncational and mutational analyses of the functional domains of CnaA to investigate the relevance of these domains for localization and function of CnaA at the septum. Importantly we found that (i) CaM, the key protein known to activate calcineurin is not required for septal localization of CnaA but is required for its function at the hyphal septum, (ii) the PxIxIT substrate binding motif in CnaA is required for its localization at the hyphal septum, indicating it localizes at the septum by interacting with other as yet unknown protein/s(iii) binding of CnaB subunit is not necessary for septal localization of CnaA but the regulatory subunit is required for its activation at the septum, and (iv) triple mutations in the catalytic active site do not affect septal localization of CnaA but completely block hyphal growth revealing that both septal localization and activity of CnaA are required for proper hyphal growth.

75. Calcium imaging and measurement during growth and response to stresses in *Aspergillus fumigatus*. Alberto Muñoz[1], Margherita Bertuzzi[2], Jan Bettgenhaeuser[1], Elaine Bignell[2], Nick Read[1]. 1) Fungal Cell Biology Group, University of Edinburgh, Edinburgh, United Kingdom; 2) Microbiology Section, Imperial College London, London, United Kingdom.

Calcium signalling and homeostasis are essential for the growth, differentiation and virulence of filamentous fungi. During infection, *A. fumigatus* must balance concomitant demands to: (1) withstand toxic levels of exogenous calcium (3-5 mM) in the host environment which can be >100,000x that of the fungal cytosolic free calcium ($[Ca^{2+}]_c$) concentration; (2) appropriately integrate homeostatic and stress-responsive adaptations; and (3) undergo normal calcium signalling. There is evidence for calcium signalling regulating numerous processes including spore germination and hyphal tip growth. The low resting level of $[Ca^{2+}]_c$ (50-100 nM) is maintained by Ca^{2+}-pumps and -antiporters, and cytoplasmic Ca^{2+}-buffering. However, $[Ca^{2+}]_c$ becomes an intracellular signal when its concentration is transiently increased. We have developed two methods for measuring and imaging $[Ca^{2+}]_c$: (1) 96-well plate luminometry using the genetically encoded, bioluminescent aequorin; and (2) fluorescence microscopy using the genetically encoded calcium-sensitive, fluorescent protein G-CaMP5. Aequorin is ideally suited for quantitative measurements of $[Ca^{2+}]_c$ calcium signatures in cell populations whereas fluorescence imaging of the G-CaMP5 is good for single cell and subcellular measurements of $[Ca^{2+}]_c$. Using the aequorin methodology we have found that transient increases in $[Ca^{2+}]_c$ with specific, reproducible calcium signatures in *A. fumigatus* arise from exposure to stresses such as high external calcium. In our analysis, $[Ca^{2+}]_c$ spikes in actively growing hyphal tips have been imaged using G-CaMP5. Exposure of conidial germlings to high external calcium induces dramatic and very dynamic changes in $[Ca^{2+}]_c$ with the generation of localized $[Ca^{2+}]_c$ transients and waves. Furthermore, there is considerable heterogeneity in the $[Ca^{2+}]_c$ responses of different germlings within the cell population. Calcium imaging and measurement using genetically encoded probes, particularly when combined with pharmacological and genetic analyses, will provide major new insights into calcium signalling in filamentous fungi.

76. WITHDRAWN

77. The copper transporter *ctpA* in *Aspergillus fumigatus* is critical for conidial melanization and virulence in an invertebrate infection model. Srijana Upadhyay, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Aspergillus fumigatus is an opportunistic pathogen that causes life-threatening invasive diseases in immunocompromised hosts. This fungus produces abundant, easily aerosolized, and heavily melanized conidia that are the infectious particles. The melanin, or the bluish green pigment coated on the conidial surface, is associated with fungal virulence and resistance to environmental stresses. This melanin is synthesized through the DHN melanin pathway by a cluster composed of six structural biosynthetic genes. Although all *Aspergillus* species produce conidial melanin, this DHN melanin gene cluster found in *A. fumigatus* is not conserved in all species of this genus. In other species, laccases are critical for melanization and copper has been shown to be critical for their activity. In *A. nidulans*, defective *ygA* that encodes a copper transporter results in reduction in conidial laccase activity and poor conidial pigmentation. Whether copper is important for conidial melanization or whether it affects the function of the DHN gene cluster in *A. fumigatus* are not clear. In this study we have identified *ctpA* in *A. fumigatu*s as the homolog of *ygA* in A nidulans and demonstrated its importance for conidial melanization under the copper limiting and the copper replete conditions. The defect in melanization caused by the deletion of the *ctpA* gene can be remediated by addition of copper in the media or by the overexpression of the *ctpA* gene. Lack of melanin is caused by growing the wild type in the copper-limiting conidiation or by the deletion of the *ctpA* gene. This renders the *A. fumigatus* conidia more immune-dominant, since these conidia can cause exacerbated immune-responses from the invertebrate host, larvae of *Galleria mellonella*. Furthermore, we have identified and characterize regulators that play important roles in maintaining copper homeostasis and melanization in *A. fumigatus*.

78. *Aspergillus nidulans* SNXA^{HRB1} is an SR/RRM family protein that rescues defects in the CDC2/CYCLINB pathway. Steven James[1], Travis Banta[2], James Barra[1], Clifford Coile[2], Ryan Day[2], Cheshil Dixit[2], Steven Eastlack[2], Anh Giang[2], Yulon Huff[2], Julie Kobie[1], Faustin Mwambutsa[2], Mimi Nguyen[2], Amanda Orzechowski[1], Kristin Shingler[1], Sarah Lea Anglin[2]. 1) Dept. Biology, Gettysburg College, Gettysburg, PA; 2) Dept. Biology, Millsaps College, Jackson, MS.

Control of the eukaryotic G2/M transition by CDC2/CYCLINB is tightly regulated. To further characterize this regulation in *Aspergillus nidulans*, we conducted a screen for extragenic suppressors of *nimX*2^{cdc2} that resulted in the identification of the cold-sensitive, G1-arresting *snxA*1 mutation. Our data show that *snxA*1 suppresses defects in regulators of the G2/M transition, including *nimX*2^{cdc2}, *nimE*6^{cyclinB}, and *nimT*23^{cdc25}, but does not suppress the G1/S-arresting *nimE*10^{cyclinB} mutation or any of four S phase mutations. Furthermore, the *snxA*1 mutation or deletion of *snxA* alter localization patterns of NIME^{CYCLINB} at the restrictive temperatures for *snxA*1 and *nimX*2, supporting a role for SNXA in cell cycle control. *snxA* encodes the *A. nidulans* ortholog of

Saccharomyces cerevisiae Hrb1/Gbp2, nonessential shuttling mRNA binding proteins belonging to the SR (Serine-Arginine Rich) and RRM (RNA Recognition Motif) protein family. *snxA*^{hrb1} is nonessential, its deletion phenocopies the *snxA*1 mutation, and overexpression of gDNAs or of alternatively spliced *snxA* cDNAs rescues *snxA*1 mutant phenotypes. SNXA^{HRB1} is predominantly nuclear, but is not retained in the nucleus during the partially-closed mitosis of *A. nidulans*. We further demonstrate that the *snxA*1 mutation does not suppress *nimX*2 by altering NIMX2^{CDC2}/NIME^{CYCLINB} kinase activity, suggesting that the effects of SNXA1 on NIMX2^{CDC2}/NIME^{CYCLINB} may be due to altered localization of NIME^{CYCLINB}. These data suggest a novel role in G2/M regulation for this SR/RRM family member. This work was supported by the Mississippi INBRE funded by grants from the National Center for Research Resources (5P20RR016476-11) and the National Institute of General Medical Sciences (8 P20 GM103476-11) from the National Institutes of Health.

79. The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. Oezguer Bayram[1*], Oezlem Sarikaya Bayram[1], Yasar Luqman Ahmed[2], Jun-Ichi Maruyama[1,4], Oliver Valerius[1], Silvio Rizzoli[3], Ralf Ficner[2], Stefan Irniger[1], Gerhard Braus[1]. 1) Institute of Microbiology & Genetics, Department of Molecular Microbiology and Genetics, Georg-August-Universität, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Molecular Structural Biology, Institute for Microbiology and Genetics, Georg-August-Universität, Goettingen; 3) European Neuroscience Institute, Deutsche Forschungsgemeinschaft Center for Molecular Physiology of the Brain/Excellence Cluster 171, 37077 Göttingen; 4) Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from the plasma membrane to the nucleus. We show here that the module of the filamentous fungus Aspergillus nidulans (An) consists of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11, and the AnSte50 adaptor. The fungal MAPK module controls the coordination of fungal development and secondary metabolite production. It lacks the membrane docking yeast Ste5 scaffold homolog but similar to yeast the entire MAPK module interacts with each other at the plasma membrane. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 interacts with the conserved nuclear transcription factor AnSte12 to initiate sexual development and phosphorylates VeA which is a major regulatory protein required for sexual development and coordinated secondary metabolite production. Our data suggest that not only Fus3 but even the entire MAPK module complex of four physically interacting proteins can migrate from plasma membrane to nuclear envelope.

80. Functional analysis of sterol transporter in filamentous fungus Aspergillus nidulans. Nicole Bühler, R. Fischer, N. Takeshita. Microbiology, Karlsruhe Institut of Technology, Karlsruhe, Germany.

A continuous flow of secretion vesicles from the hyphal cell body to the growing hyphal tip provides the delivery of proteins and lipids to the tip and is essential for cell wall and cell membrane extension at the tip. Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. Although the importance of SRDs is becoming clear, their exact roles and formation mechanisms remain rather unclear. Transport of sterol to hyphal tips is thought to be important for the SRDs organization. Oxysterol binding proteins, which are conserved from yeast to human and involved in vesicular trafficking, signalling, lipid metabolism and non-vesicular sterol transport. Saccharomyces cerevisiae has seven oxysterol binding protein homologues (OSH1-7). Their subcellular distributions are regulated respectively. The OSH proteins are thought to function as a sterol transporter between closely located membranes independently of vesicle transport. In the filamentous fungus Aspergillus nidulans, we found five OSH genes. To investigate their functions for the polarized growth and SRDs organization, their localization are analyzed by GFP tagging. The gene-deletion strains are constructed and analyzed. Their expression levels are analyzed via qRT-PCR.

81. Mechanisms of cellular resistance to copper and arsenic in Aspergillus nidualans. Steven H. Denison. Natural Sciences, Eckerd College, St Petersburg, FL.

Copper is an essential element for cells that is toxic in high concentrations. Understanding cellular mechanisms for survival in high concentrations of copper is important for at least two reasons. Firstly, copper is an important environmental contaminant. In addition, two human genetic disorders, Wilson and Menkes diseases, result from impaired copper transport. I am using the filamentous fungus, Aspergillus nidulans, as a model organism for understanding cellular mechanisms of resistance to high concentrations of copper. I identified a gene in A. nidulans homologous to the copper transporting ATPase-encoding genes mutated in Wilson and Menkes diseases. To determine the location of this copper transporter in A. nidulans cells, I used fusion PCR to construct a GFP-tagged version of the gene, which was then transformed into A. nidulans in a gene replacement. In terms of its location in the cell, the A. nidulans GFP-tagged protein behaves in the same way as the Menkes disease protein: it is located to an intracellular compartment (possibly the Golgi, as in human cells) in low copper medium but appears in the plasma membrane upon addition of excess copper to the medium. In addition, disruption of the A. nidulans copper transporter gene results in increased sensitivity to copper in the growth medium relative to wild type cells and cells expressing the GFP-tagged protein. Taken together, these data suggest that the transporter functions in the plasma membrane in high copper environments to remove excess copper from cells. Arsenic is also an important environmental contaminant. To begin to understand the mechanism of arsenic resistance in A. nidulans cells, I have GFP-tagged and disrupted a putative arsenic transporter gene from A. nidulans. Disruptants are more sensitive to arsenic than wild type cells and cells expressing the GFP-tagged protein. The GFP fusion protein localizes to the plasma membrane, consistent with a function for the protein in transporting arsenic across the plasma membrane, removing arsenic from cells.

82. Functional characterization of *Aspergillus nidulans* ANID_05595.1: a possible homologue of the polarisome component Pea2. Nathan W Gross, Bradley Downs, Steven D Harris. Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68588-0660.

Cell polarity is a defining feature of filamentous fungal growth. However, the complete molecular pathway that regulates this morphogenetic characteristic has not yet been elucidated. In *Aspergillus nidulans*, a germ tube emerges from a discrete location along the conidium following a brief period of isotropic swelling. Plasma membrane and cell wall components are continuously added to the apex of the germ tube via microtubule and actin mediated trafficking of vesicles to this region. As growth progresses, germ tube cells undergo cytokinesis and are compartmentalized by septa. Additionally, the cell wall becomes increasingly cross-linked throughout subapical regions forming a hypha, which continues to grow in the same polarized manner. ANID_05595.1 is located on chromosome 5, contig 96, and encodes a 946 amino acid hypothetical involucrin repeat protein. To investigate the function of ANID_05595.1 in *A. nidulans*, deletion mutants were generated using *pyrG* from *Aspergillus fumigatus* as a selectable marker. This mutation resulted in restricted colony growth, increased hyphal diameter, and dichotomous hyphal branching patterns. These phenotypes suggest that ANID_05595.1 function is important to the maintenance of polarized cell growth in *A. nidulans* and other ascomycetes. The hypothetical ANID_05595.1 protein shares characteristics with *Saccharomyces cerevisiae* Pea2, a polarisome component required for bipolar budding and mating. Along with structural similarities, the phenotypes observed in *S. cerevisiae* D*Pea2* are similar to *A. nidulans* D5595. This suggests that ANID_05595.1 may perform a similar mechanistic function to Pea2 in *A. nidulans*.

83. *Aspergillus nidulans* septin interactions and post-translational modifications. Yainitza Hernandez-Rodriguez[1], Shunsuke Masuo[2], Darryl Johnson[3], Ron Orlando[3,4], Michelle Momany[1]. 1) Plant Biology, University of Georgia, Athens, GA, US; 2) Laboratory of Advanced Research A515, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, JP; 3) Department of Chemistry, University of Georgia, Athens, GA, US; 4) Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, US.

Septins are cytoskeletal elements found in fungi, animals, and some algae, but absent in higher plants. These evolutionarily conserved GTP binding proteins form heteroligomeric complexes that seem to be key for the diverse cellular functions and processes that septins carry out. Here we used *Aspergillus nidulans*, a model filamentous fungus with well defined vegetative growth stages to investigate septin-septin interactions. *A. nidulans* has five septins: AspA/Cdc11, AspB/Cdc3, AspC/Cdc12 and AspD/Cdc10 are orthologs of the "core-filament forming-septins" in *S. cerevisiae*; while AspE is only found in filamentous fungi. Using S-tag affinity purification assays and mass spectrometry we found that AspA, AspB, AspC and AspD strongly interact in early unicellular and multicellular vegetative growth. In contrast, AspE appeared to have little or no interactions with core septins in unicellular stages before septation. However, after septation AspE interacted with other septins, for which we postulate an accessory role. AspE localized to the cortex of actively growing areas and to septa, and localizations are dependent on other septin partners. Interestingly, core septin localizations can also depend on accessory septin AspE, particularly post-septation. In addition, LC-MS/MS showed acetylation of lysine residues in AspA before septation and AspC after septation. Western blot analysis using an anti-acetylated lysine antibody showed that AspC is highly acetylated in all stages examined, while other septins showed acetylation post-septation. Though LC-MS analysis failed to detect phosphorylation of septins, this modification has been widely reported in fungal septins. Using phosphatase treatments and Western Bloting, we found phosphorylation of AspD, but no other septins. This is interesting because AspD belongs to a special group of septins that lack a C-terminal coiled-coil found in other septins. However, septin localization is not affected by the absence of AspD/Cdc10, but by the absence of filamentous fungi specific septin AspE. Our data suggests that septin interactions and modifications change during development and growth in *A. nidulans*, and that some modifications are septin specific.

84. A highly conserved sequence motif is required for PkcA localization to septation sites and protein function in *Aspergillus nidulans*. Loretta Jackson-Hayes[1], Terry Hill[1], Darlene Loprete[1], Claire DelBove[1], Omolola Dawodu[2], Jordan Henley[3], Ashley Poullard[3], Justin Shapiro[1]. 1) Rhodes College, Memphis, TN 38112; 2) Rust College, Holly Springs, MS 38635; 3) Tougaloo College, Tougaloo, MS 39174.

Many proteins with diverse functions contribute to cell wall synthesis in polarized growth and septation. Some of these proteins play similar roles at tips and septa, while others are exclusively involved in one process or the other. In *Aspergillus nidulans*, wild type protein kinase C (PkcA) localizes to growing hyphal tips and septation sites, and a role for PkcA in cell wall synthesis is supported by the inability of PkcA mutant strains to exhibit resistance to cell wall perturbing agents. PkcA localization to septation sites is dynamic. Upon initiation of septum formation PkcA is organized as a ring at periphery of the septation site. The ring constricts in synchrony with the actin/myosin contractile ring and dissipates when septa are fully matured. To determine which domains are important for septum site localization, green fluorescent protein tagged, domain-deleted versions of PkcA were constructed. The domains that are vital to *A. nidulans* maintenance of cell wall integrity were separately identified by growing the domain deleted stains in the presence of the cell wall stressor calcofluor white. We have determined that the localization signal and the domain responsible for resistance to calcofluor white are distinct. The PkcA septation site localization signal is found within a region having homology with C2 domains of PKC proteins found in other organisms. Observations of both *N*- and *C*- terminal truncations support the conclusion that the PkcA septation site localization signal lies within the final 20 amino acids of the C2 domain. Removal of these amino acids causes PkcA mislocalization to the cytoplasm. Furthermore, removal of the localization signal renders the resulting truncated proteins less able to complement calcofluor white hypersensitivity in a strain carrying a mutation in its *PkcA* gene, highlighting the requirement of proper localization for this aspect of PkcA function.

85. The MpkB MAP kinase plays a role in autolysis and conidiation of *Aspergillus nidulans*. Ji Young Kang[1], Keon-Sang Chae[2], Dong-Min Han[3], Kwang-Yeop Jahng[1]. 1) Dept Biol, Chonbuk Natl Univ Col Nat Sci, Jeonju, Jeonbuk, South Korea; 2) Dept Mol Biol, Chonbuk Natl Univ Col Nat Sci, Jeonju, Jeonbuk, South Korea; 3) Div Life Sci, Wonkwang Univ, Iksan, Jeonbuk, South Korea.

The *mpkB* gene of *Aspergillus nidulans* encodes a MAP kinase homologous to Fus3p of *Saccharomyces cerevisiae* which is involved in conjugation process. MpkB is required for accomplishing successfully the sexual development at the anastomosis and post-karyogamy stages. The *mpkB* deletion strain produced conidia under the repression condition of conidiation such as sealing in the dark and even in the submerged culture concomitant with persistent *brlA* expression, implying that MpkB might have a role in timely regulation of *brlA* expression. The deletion of *mpkB* caused hyphal fragmentation, disorganization of mycelial balls and dry cell mass reduction in the submerged culture as well as the *chiB*, *mutA* and *pepJ* genes which are encoding cell wall hydrolytic enzymes to be transcribed highly in the culture. These results suggest that MpkB might play a role in regulation of BrlA-involving autolysis.

86. Beyond green mining: analysis of fungal cytochemistry using gold nanoparticles. Fatemeh Farazkhorasani[1], Martin Prusinkiewicz[2], Kathleen M Gough[1], Susan GW Kaminskyj[2]. 1) Chemistry, University of Manitoba, Winnipeg, Canada; 2) Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Cells including fungal hyphae and other microorganisms, as well as fungal growth medium including both complex and defined composition, can reduce solutions of $HAuCl_4$ to elemental gold nanoparticles (AuNPs). As described in 2012 Analyst 137:4934-42, we have shown that AuNPs formed by growing fungal hyphae can be used as analytical substrates for surface-enhanced Raman scattering (SERS) spectroscopic analysis. These SERS spectra are in the same energy range as our Fourier-transform infrared (FTIR) spectroscopic studies that provided information about cell composition. However, SERS is orders of magnitude more sensitive, and analysis is limited to cell components within a few nanometers of the AuNP. Our current interest is the fungal cell wall, which forms a porous interface between the cell and its environment. Cell wall chemistry is intrinsically related to cell-environment interactions, particularly for pathogenesis. The fungal wall is about 25 % of fungal dry weight, and its synthesis and maintenance is estimated to require ~25 % of the fungal genome. Fungal walls are ~ 80 % carbohydrate. Minor structural differences in carbohydrate bonding can cause profound changes in their metabolism, which complicates analysis. Preliminary studies described in the Analyst paper showed that SERS-active AuNPs can be generated by living hyphae. Higher Au concentrations produced larger AuNPs within and on the hypha, but in addition were lethal within 30 min. Lower Au concentrations produced clusters of smaller AuNPs on the cell wall surface, and were not lethal. These were also SERS-active. We are using SERS to probe the wall composition of engineered mutants in the Aspergillus galactofuranose biosynthesis pathway, which plays key roles in fungal growth and drug resistance. We expect the combination of fungal genetic engineering and high sensitivity/high spatial-resolution chemical analysis will provide novel information about fungal growth and infectivity.

87. *Aspergillus nidulans* as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug

resistance mutations. Xiaoxiao Sean He, Shengnan Jill Li, Susan Kaminskyj. Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Systemic fungal infections are estimated to contribute to ~10% of hospital deaths. Systemic fungal infections are most dangerous for the young, the old, and the already sick, since their immune systems are less vigorous. Most antifungal drugs in current clinical use target ergosterol (polyenes) or the ergosterol biosynthetic pathway (azoles and allylamines). Drugs against beta-glucan synthesis (echinocandins) are effective against aspergillosis and candidaisis. The use of compounds that target fungal enzymes inevitably leads to the development and natural selection of drug resistant fungal strains. Not only are the anti-fungal drugs in current clinical use losing efficacy in some situations, but in addition the high level of conservation between animal and fungal physiology leaves relatively few relevant targets to explore. However, it is likely that for any drug-enzyme combination there will be relatively few mutations that could increase drug resistance while still maintaining enzyme function. We are using *Aspergillus nidulans* as an experimental model system to assess the number and identity of mutations that lead to drug resistance. As proof of concept, we grew wild type *A. nidulans* on replicate plates containing a sub-lethal concentration of Calcofluor. These developed fast-growing sectors beginning at ~ 5 d (70 rounds of mitosis). Preliminary results show that many of these sectors harboured heritable, single-gene mutations. To date, mutated genes that confer robust, heritable resistance to Calcofluor that were identified by next generation sequencing have roles in cell wall synthesis, cell wall integrity regulation, or drug detoxification. We suggest this strategy will be useful for predicting genetically-mediated anti fungal resistance adaptation and help us to be ahead in the drug-resistance arms race.

88. *Aspergillus nidulans* cell walls lacking galactofuranose are more susceptible to glucan degrading enzymes. Biplab Paul[1], Tanya Dahms[1], Susan Kaminskyj[2]. 1) Dept Chemistry and Biochemistry, Univ Regina, Regina, SK, Canada; 2) Dept Biology, Univ Saskatchewan, Saskatoon, SK, Canada.

The cell wall of *Aspergillus* is a dynamic organ, consisting of a semi-permeable network of mannoprotein, and alpha- and beta-glucans. These components are remodeled as fungal cell grows and responds to its environments. By weight, fungal walls are estimated to be 35-45% alpha-(1,3)-glucan, 20-35% beta-(1,3)-glucan, 20-25% galactomannan, 7-15% chitin (beta-1,4-glucan), and 4% beta-(1,6)-glucan. Evidence from literature sources suggest that the *Aspergillus* wall 'core' is chitin and galactomannan linked to beta-1,6- and beta-1,6-glucan. Galactofuranose (Gal-f) appears to play a central role in *Aspergillus* cell wall maturation. Previously, we showed that Gal-f biosynthesis is important for wild type chemical, physical, structural properties of the *A. nidulans* cell wall. We propose that the lack of Gal-f disrupts the proper packing of cell wall components, giving rise to more disordered surface subunits and so to greater deformability. Here, we show results from an investigation of the susceptibility of *Aspergillus* Gal-f biosynthesis deletion strains to glucan degrading enzyme using atomic force microscopy. Topographic images of glucanase- and laminarinase-treated wildtype strains suggest that glucan is at least one component of the cell surface subunits. Strains that lacked Gal-f were more susceptible to beta-1,3-glucanase.

89. The GATA-type transcription factor NsdD is a key regulator of conidiation and secondary metabolism in *Aspergillus*. Mi-Kyung Lee[1], Nak-Jung Kwon[1], Im-Soon Lee[2], Jae-Hyuk Yu[1]. 1) Bacteriology, University of Wisconsin Madison, MADISON, WI, USA; 2) Department of Biological Sciences, Konkuk University, Seoul, Republic of Korea.

Asexual development (conidiation for higher fungi) is the most common reproductive mode of many fungi; yet, its regulatory mechanisms remain to be understood. In this study, we carried out a multi-copy based genetic screen in the absence of the repressor of conidiation *sfgA*, which is designed to identify a new set of negative regulator(s) of conidiation. Among over 100,000 colonies, 45 transformants showing altered conidiation were isolated, of which 10 defined the *nsdD* gene (AN3152), a key activator of sexual fruiting. The others have defined AN7507, AN2009, AN1652, AN5833 and AN9141. A series of verification, genetic and mycotoxin analyses revealed that only NsdD is a true negative regulator of *brlA* (an essential activator of conidiation) and conidiation, and that NsdD acts downstream of *fluG* and *flbA~E*, but upstream of *brlA*. The removal of NsdD was sufficient to cause hyper-active conidiation even in liquid submerged culture, as well as early and prolonged activation of *brlA*, suggesting that NsdD is indeed a key repressor of *brlA* and conidiation. Moreover, the deletion of *nsdD* results in hyper-active conidiation and altered production of mycotoxins in the opportunistic human pathogen *Aspergillus fumigatus* and the aflatoxin-producing human/plant pathogen *Aspergillus flavus*. Importantly, we have discovered that *nsdD* encodes two differentially expressed mRNAs and polypeptides (b and a). Finally, the subsequent transient promoter analysis using the *brlA* promoter::luciferase fusion constructs have revealed that NsdD negatively regulates the *brlAb* promoter activity. In summary, NsdD is a key negative regulator of conidiation acting direct upstream of *brlA* in A. *nidulans*, and couples conidiation and mycotoxin biosynthesis in *Aspergilli*.

90. THE *velvet* regulators in *Aspergilli*. Heesoo Park, JJae-Hyuk Yu. Bacteriology, University of Wisconsin Madison, Madison, WI.

The *velvet* regulators are the key players coordinating fungal growth, differentiation and secondary metabolism in response to various internal and external cues. All *velvet* family proteins contain the conserved *velvet* homology motif (~190 a.a.), and define a novel class of fungal specific transcription factors with the DNA binding ability. Some *velvet* regulators form time and/or cell type specific complexes with other *velvet* regulators or non-*velvet* proteins. These complexes play differential roles in regulating growth, development, sporogenesis and toxigenesis. Among the *velvet* complexes, the VelB-VosA hetero-complex acts as a functional unit conferring the completion of sporogenesis (focal trehalose biogenesis and spores wall completion), and the long-term viability of spore, and the attenuation of conidial germination in the model filamentous fungus *Aspergillus nidulans*. Both *velB* and *vosA* are activated by AbaA in developing cells, and the VelB-VosA complex plays a dual role in activating genes associated with spore maturation and in exerting negative feedback regulation of developmental genes. Interestingly, the VelB-VosA complex plays similar yet somewhat distinct roles in spore maturation, dormancy and germination in *Aspergillus fumigatus* and *Aspergillus flavus*. A comprehensive model depicting the roles of the velvet regulators in aspergilli is presented.

91. Coordinated regulation of asexual development, cell death and autolysis by the C2H2 zinc finger transcription factor BrlA in *Aspergillus nidulans* . István Pócsi[1], Jae-Hyuk Yu[2], Tamás Emri[1]. 1) Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Debrecen, Hungary; 2) Departments of Bacteriology and Genetics, University of Wisconsin, Madison, WI, USA.

Carbon starvation elicited in submerged cultures of *Aspergillus nidulans* triggers all various physiological responses affecting cell wall composition, stress tolerance, protein synthesis and primary and secondary metabolisms. Particularly, function of vacuoles and endoplasmic reticulum is drastically affected leading to the re-utilization of cellular biopolymers through macroautophagy and the removal of damaged cells by apoptosis. Autolytic cell wall degradation is also an integrant part of this highly complicated and delicate regulatory process. Importantly, although the development of conidiophores is initiated in carbon-starving submerged cultures, these structures are underdeveloped and only simple conidia are observable. There is an increasing body of evidence supporting the idea that the transcription factor BrlA, a well-studied central regulator of conidiation in aspergilli, is one of the most important master controllers orchestrating development and autolysis in submerged culture of aspergilli. Major processes subjected to BrlA-dependent regulation under these conditions include the production of autolytic enzymes, rodlet proteins and melanins. In fungal biology, the concerted and well-balanced regulation of conidiogenesis, cell death and autolysis is of primary importance because any overproduction of cell wall hydrolases may affect cell vitality and colony propagation rather disadvantageously. The age-dependent production of autolytic hydrolases coincides with the synthesis of antimicrobial

metabolites and proteins, and all these carbon-starvation-associated products will affect markedly the microbiome in the ecological niche the autolyzing fungus occupies. A deeper understanding of the BrlA-mediated spatial and temporal regulatory mechanisms for conidiogenesis, cell death and autolysis may lead to the development of new industrial strains for heterologous protein production and/or novel biocontrol technologies.

92. Whole-genome sequencing identifies novel alleles of genes required for organelle distribution and motility in *Aspergillus nidulans*. Kaeling Tan, Anthony Roberts, Martin Egan, Mark Chonofsky, Samara Reck-Peterson. Cell Biology, Harvard Med Sch, Boston, MA.

Many organelles are transported long distances along microtubules in eukaryotic organisms by dynein and kinesin motors. To identify novel alleles and genes required for microtubule-based transport, we performed a genetic screen in the filamentous fungus, *Aspergillus nidulans*. We fluorescently-labeled three different organelle populations known to be cargo of dynein and kinesin in *Aspergillus*: nuclei, endosomes, and peroxisomes. We then used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Using whole-genome sequencing, we found a number of single nucleotide polymorphisms (SNPs) that resulted in misdistribution of peroxisomes, endosomes, or nuclei. Some of these SNPs were novel alleles of cytoplasmic dynein/ *nudA*, Arp1/ *nudK* (dynactin), Lis1/ *nudF*, and kinesin-1/ *kinA*. Here, we characterize the *in vivo* transport defects in these novel mutants and analyze the single molecule *in vitro* motility properties of purified mutant motor proteins. We also describe our methods for using whole genome sequencing as a tool in mutagenesis studies in *A. nidulans*.

93. Two methyltransferase protein complexes control fungal development and secondary metabolite production. Oezlem Sarikaya Bayram[1], Oezguer Bayram[1], Jong-Hwa Kim[2], Keon-Sang Chae[3], Dong-Min Han[4], Kap-Hoon Han[2], Gerhard Braus[1]. 1) Institute of Microbiology & Genetics, Dept. of Molecular Microbiology and Genetics, Georg August University, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, Korea; 3) Division of Biological Sciences, Chonbuk National University, Jeonju, 561-756, Korea; 4) Division of Life Sciences, Wonkwang University, Iksan, 570-749, Korea.

Coordination of development and secondary metabolism of the filamentous fungus *Aspergillus nidulans* requires the trimeric velvet complex consisting of VelB-VeA and the putative methyltransferase LaeA. We discovered a second trimeric protein complex for the same control mechanism consisting of an unusual zinc finger domain protein and even two subunits containing canonical methyltransferase domains. In contrast to velvet, which is assembled in the nucleus, the novel trimeric protein complex is formed at the plasma membrane. Functional green fluorescent protein fusions revealed that both methyltransferases are released from the membrane-bound zinc finger domain and migrate to the nucleus. The dimeric nuclear methyltransferase complex physically interacts with chromatin factors as heterochromatin protein and has an impact on the expression of asexual or sexual developmental genes as well as secondary metabolite gene clusters. Consistently, deletions of the corresponding genes result in defects in light response. Our results support that a trimeric membrane complex initiates a signalling pathway which is mediated by two methyltransferases which transduce the signal to nuclear chromatin and affect gene expression. The interplay between the novel methyltransferase complex and the velvet complex remains to be elucidated.

94. Control of Multicellular Development by the Physically Interacting Deneddylases DEN1/DenA and COP9 Signalosome. Josua Schinke[1], Martin Christmann[1], Tilo Schmaler[2], Colin Gordon[3], Xiaohua Huang[2], Özgür Bayram[1], Sina Stumpf[1], Wolfgang Dubiel[2], Gerhard Braus[1]. 1) Microbiology and Genetics, Georg-August-University, Göttingen, Niedersachsen, Germany; 2) Department of General, Visceral, Vascular and Thoracic Surgery, Division of Molecular Biology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany; 3) MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

Deneddylases remove the ubiquitin-like protein Nedd8 from modified proteins. An increased deneddylase activity has been associated to various human cancers. In contrast, we show here that a mutant strain of the model fungus Aspergillus nidulans which is deficient in two deneddylases is viable but can only grow as a filament and has lost most of the potential for multicellular development. The DEN1/DenA and the COP9 signalosome (CSN) deneddylases physically interact in A. nidulans as well as in human cells, and CSN targets DEN1/DenA for protein degradation. Fungal development responds to light and requires both deneddylases for an appropriate light reaction. In contrast to CSN which is necessary for sexual development, DEN1/DenA is required for asexual development. The CSN-DEN1/DenA interaction which affects DEN1/DenA protein levels presumably balances cellular deneddylase activity. A deneddylase disequilibrium impairs multicellular development and suggests that control of deneddylase activity is important for multicellular development.

95. Visualization of apical membrane domains in *Aspergillus nidulans* by Photoactivated Localization Microscopy (PALM). Norio Takeshita[1], Yuji Ishitsuka[2], Yiming Li[2], Ulrich Nienhaus[2], Reinhard Fischer[1]. 1) Dept. of Microbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Institute for Applied Physics, Karlsruhe Institute of Technology.

Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The size of SRDs is around a few mm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs were analyzed by super-resolution microscope technique, Photoactivated Localization Microscopy (PALM). Membrane domains were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. Size, number, distribution and dynamics of membrane domains, and dynamics of single molecules were investigated. Time-laps analysis revealed the dynamic behavior of exocytosis.

96. Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function. Lakshmi Preethi Yerra, Steven Harris. University of Nebraska-Lincoln, Lincoln, NE.

In the filamentous fungus Aspergillus nidulans, the transition from hyphal growth to asexual development is associated with dramatic changes in patterns of cellular morphogenesis and division. These changes enable the formation of airborne conidiophores that culminate in chains of spores generated by repeated budding of phialides. Our objective is to characterize the regulatory modules that mediate these changes and to determine how they are integrated with the well-characterized network of transcription factors that regulate conidiation in A. nidulans. Because protein phosphorylation is likely to be a key component of these regulatory modules, we have exploited the availability of A. nidulans post-genomic resources to investigate the roles of protein kinases and phosphatases in developmental morphogenesis. We have used the protein kinase and phosphatase deletion mutant libraries made available by the Fungal Genetics Stock Center to systematically screen for defects in conidiophore morphology and division patterns. Our initial results implicate ANID_11101.1 (=yeast Hsl1/Gin4) in phialide morphogenesis, and also reveal the importance of ANID_07104.1 (=yeast Yak1) in the maintenance of cell integrity during asexual development. Additional deletion mutants with reproducible defects have been identified and will be described in detail. We will also summarize initial results from double mutant analyses that attempt to place specific protein kinase deletions within the regulatory network that controls conidiation.

97. The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in *Aspergillus*. Nak-Jung Kwon[1], Hee Soo Park[2], Seunho Jung[3], Sun Chang Kim[4], Jae-Hyuk Yu[1,2]. 1) Dept Bacteriology, University of Wisconsin, Madison, WI. USA; 2) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, USA,; 3) Department of Bioscience and Biotechnology, and Center for Biotechnology Research in UBITA, Konkuk University, Seoul, Republic of Korea; 4) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Dae-Jon, Republic of Korea.

Heterotrimeric G proteins (G proteins) govern growth, development, and secondary metabolism in various fungi. Here, we characterized *ricA*, which encodes a putative GDP/GTP exchange factor for G proteins in the model fungus *Aspergillus nidulans* and the opportunistic human pathogen *Aspergillus fumigatus*. In both species, *ricA* mRNA accumulates during vegetative growth and early developmental phases, but it is not present in spores. The deletion of *ricA* results in severely impaired colony growth and the total (for *A. nidulans*) or near (for *A. fumigatus*) absence of asexual sporulation (conidiation). The overexpression (OE) of the *A. fumigatus ricA* gene (*AfricA*) restores growth and conidiation in the D*AnricA* mutant to some extent, indicating partial conservation of RicA function in *Aspergillus*. A series of double mutant analyses revealed that the removal of RgsA (an RGS protein of the GanB Ga subunit), but not *sfgA*, *flbA*, *rgsB*, or *rgsC*, restored vegetative growth and conidiation in *AnricA*. Furthermore, we found that RicA can physically interact with GanB in yeast and *in vitro*. Moreover, the presence of two copies or OE of *pkaA* suppresses the profound defects caused by D*AnricA*, indicating that RicA-mediated growth and developmental signaling is primarily through GanB and PkaA in *A. nidulans.* Despite the lack of conidiation, *brlA* and *vosA* mRNAs accumulated to normal levels in the *ricA* mutant. In addition, mutants overexpressing *fluG* or *brlA* (OE*fluG* or OE*brlA*) failed to restore development in the *AnricA* mutant. These findings suggest that the commencement of asexual development requires unknown RicA-mediated signaling input in *A. nidulans*.

98. Evidence for a role of peroxisomes in microtubule organization. Ying Zhang, Andreas Herr, Reinhard Fischer. Karlsruhe Institute of Technology, Karlsruhe, Germany.

In *Aspergillus nidulans* spindle pole bodies (SPBs) and septum-associated microtubule-organizing centres (sMTOCs) polymerize cytoplasmic microtubules. Previously, we identified a novel MTOC-associated protein, ApsB (*Schizosaccharomyces pombe* mto1), whose absence affected MT formation from sMTOCs more than from SPBs, suggesting that the two protein complexes are organized differently (Suelmann et al., 1998; Veith et al., 2005). In addition, we discovered that ApsB localizes to a subclass of peroxisomes apparently without a peroxisomal targeting motif. However, we found that ApsB interacts with the Woronin body protein HexA, which has a PTS1 motif at the C-terminus (Zekert er al., 2010). Our hypothesis is that ApsB is imported to peroxisomes by a piggyback import mechanism along with HexA. To further investigate the role of peroxisomes in microtubule orgnization, We created a deletion mutant of *pexC*. PexC is an essential protein for peroxisomal biogenesis (Heiland & Erdmann, 2005). The *pexC* mutant partially phenocopied the *apsB* mutant, which shows reduced sporulation and nuclear migration defects in comparison to wild type. The number of astral and cytoplasmic microtubules and the activities of sMTOCs and SPBs was reduced in the *pexC* mutant in comparison to wild type. sMTOC activity was more affected than the SPB activity, which again resembles the phenotype of the *apsB* mutant. In conclusion, peroxisomes play a role in microtubule organization through ApsB.

99. Autophagy promotes survival in aging submerged cultures of the filamentous fungus *Aspergillus niger*. Maria A. Burggraaf[1,2], Benjamin M. Nitsche[1,2], Gerda Lamers[1], Vera Meyer[2,3], Arthur F.J. Ram[1,2]. 1) Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Leiden, The Netherlands; 2) Kluyver Centre for Genomics of Industrial Fermentation, Delft, The Netherlands; 3) Institute of Biotechnology, Applied and Molecular Microbiology, Berlin University of Technology, Berlin, Germany.

The filamentous fungus *Aspergillus niger* is an important and versatile cell factory commonly exploited for the industrial-scale production of a wide range of enzymes and organic acids. Although numerous studies have been conducted aiming at improving our knowledge of degradative cellular activities that determine product yields in *A. niger* including secretion of proteases and the unfolded protein response, there is a catabolic pathway that has yet not been studied in this industrially exploited fungus, namely Autophagy. Autophagy is a well conserved catabolic process constitutively active in eukaryotes that is involved in cellular homeostasis by targeting of cytoplasmic content and organelles to vacuoles. Autophagy is strongly induced by limitation of nutrients including carbon, nitrogen and oxygen and is clearly associated with cell death. We previously demonstrated that the accumulation of empty hyphal compartments and secondary regrowth in carbon starved submerged batch cultures of *A. niger* were accompanied by a joint transcriptional induction of autophagy genes. In this study we examined the role of autophagy by deleting the *atg1*, *atg8* and *atg17* orthologues in *A. niger* and phenotypically analyzing the deletion strains in surface and submerged cultures. Our results indicate that *atg1* and *atg8* are essential for efficient autophagy whereas deletion of *atg17* has little to no effect on autophagy. Depending on the stressor, autophagy deficiency renders *A. niger* both more resistant and more sensitive to oxidative stress. Fluorescence microscopy showed that mitochondrial turnover upon carbon depletion in submerged cultures is severely blocked in autophagy impaired mutants. Furthermore, automated image analysis demonstrated that autophagy promotes survival in maintained carbon starved cultures of *A. niger*. Taken together, our results suggest that besides its function in nutrient recycling, autophagy plays important roles in physiological adaptation by organelle turnover and protection against cell death upon carbon depletion in submerged cultures.

100. Inactivation of *flbA* results in increased secretome complexity and reduced secretion heterogeneity in colonies of *Aspergillus niger*. Pauline Krijgsheld[1], Benjamin M. Nitsche[2], Harm Post[3], Ana M. Levin[1], Wally H. Müller[4], Albert J.R. Heck[3], Arthur F.J. Ram[2], A.F. Maarten Altelaar[3], Han A.B. Wösten[1]. 1) Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht,The Netherlands; 2) Department of Molecular Microbiology and Biotechnology, Institute of Biology Leiden and Kluyver Centre for Genomics of Industrial Fermentation, Leiden University, Leiden, The Netherlands; 3) Biomolecular Mass Spectrometry and Proteomics, Netherlands Proteomics Center, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands; 4) Biomolecular Imaging, Utrecht University, Utrecht, The Netherlands.

Aspergilli are among the most common fungi. They colonize substrates by secreting enzymes that degrade organic polymers into small products that can be taken up by the fungus to serve as nutrient. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the substrate is (partly) utilized in the colony center. *Aspergillus niger* is known for its capacity to secrete high amounts of proteins. Interestingly, the fungus secretes proteins in the central part and at the periphery of the colony but not in the sub-peripheral zone. The sporulating zone of the colony overlaps with the non-secreting zone, indicating that sporulation inhibits protein secretion. Indeed, strain D*flbA* that is affected early in the sporulation program secreted proteins throughout the colony. In contrast, the D*brlA* strain that still initiates but not completes sporulation did not show an altered spatial secretion. The secretome of 5 concentric zones of 7-dayold xylose-grown D*flbA* mutant colonies of *A. niger* was assessed by quantitative proteomics using stable isotope dimethyl labeling. In total 171 proteins were identified in the medium of the D*flbA* colonies, of which 33 proteins did not have a signal sequence for secretion. Out of the 138 secreted proteins, 101 had previously not been identified in the secretome of the 5 concentric zones of xylose-grown wild-type colonies. Moreover, 18 proteins had never been reported to be part of the secretome of *A. niger*. Taken together, inactivation of *flbA*, but not *brlA* results in spatial changes in secretion and in a more complex secretome. The latter may be explained by the fact that strain D*flbA* has a thinner cell wall compared to the wild type, enabling efficient release of proteins. These results can implemented in the industry to improve *A. niger* as a cell factory.

This research was financed by the Kluyver Centre for Genomics of Industrial Fermentation and by the Netherlands Proteomics Centre, which are part of the Netherlands Genomics Initiative/ Netherlands Organisation for Scientific Research.

101. Functional characterization of *A. niger* class III and class V chitin synthases and their role in cell wall integrity. Jean-Paul Ouedraogo[1], Arthur Ram[1], Vera Meyer[2]. 1) Molecular Microbiology and Biotechnology, Institut of Biology, Leiden, Netherlands; 2) Molecular and Applied Microbiology, Institut of Biotechnology, Berlin University of Technology, Berlin, Germany.

Class III and V chitin synthases play an important role in morphogenesis and cell wall integrity in many filamentous fungi. However, their function in the filamentous fungus, *A. niger* has not yet been elucidated. To address this, deletion mutants of class III and V chitin synthase-encoding genes of *A.niger*, chsB and csmB, and their role in cell wall integrity have been studied. Deficiency in conidiation and abnormal swollen conidiophores have been observed in chsB and csmB deletion mutants. Using cell wall inhibitor reagents, it was shown that the mutants are hypersensitive towards cell wall stress. However, there are differences between them as regards susceptibility to the antifungal protein AFP. These results suggest that ChsB and CsmB play an important role during asexual development and in ensuring cell wall integrity of *A. niger.* Interestingly, the data indicate that only chitin synthase csmB is important to counteract AFP inhibitory effects.

102. Exploiting transcriptomic signatures of Aspergillus niger to uncover key genes important for high protein traffic through its secretory pathway. Min Jin Kwon[1,2], Thomas Jørgensen[1], Benjamin M Nitsche[1,3], Mark Arentshorst[1], Joohae Park[1], Arthur F.J. Ram[1,2], Vera Meyer[1,3]. 1) Molecular Microbiology, Institute of Biology Leiden, Leiden, Netherlands; 2) Kluyver Centre for Genomics of Industrial Fermentation, P.O. Box 5057, 2600 GA Delft, The Netherlands; 3) Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany.

The filamentous fungus Aspergillus niger is well known for its exceptional high capacity to secrete proteins. However, system-wide insights into its secretory capacities are sparse and rational strain improvement approaches are thus limited. To gain a global view on the transcriptional basis of the secretory pathway of A. niger, we have investigated its transcriptomic fingerprint when specifically forced to overexpress the hydrolytic enzyme glucoamylase (GLA). An A. niger wild-type strain and an GLA over-expressing strain where cultivated under maltose-limited chemostat conditions. Elevated glaA mRNA and extracellular GLA levels in the over-expressing strain were accompanied by reinforced transcription of 772 genes and down-regulation of 815 genes when compared to the wild-type situation. Using GO term enrichment analysis, four higher order categories were identified in the up-regulated gene set: i) translocation, ii) protein glycosylation, iii) vesicle transport and iv) ion homeostasis. Among these, about 130 genes have predicted functions for the protein passage through the endoplasmaticum reticulum including well-known target genes of the HacA transcription factor, e.g. bipA, clxA, prpA, tigA and pdiA. To identify those genes, which are generally important for high-level secretion in A. niger, we compared the GLA transcriptome with six other secretion stress transcriptomes of A. niger, including a constitutive active HacA transcriptome, several UPR stress transcriptomes and a carbon-source induced secretion transcriptome. Overall, 40 genes were commonly up-/down-regulated under these three conditions (36 genes up-regulated, 4 down-regulated), thus defining the core set of genes important for ensuring high protein traffic through the secretory pathway.

103. Identification of two Golgi-localized putative UDP-galactofuranose transporters with overlapping function in Aspergillus niger. Joohae Park[1], Boris Tefsen[2], Ellen Lagendijk[1], Irma van Die[2], Arthur Ram[1,3]. 1) Molecular Microbiology, Institute of Biology Leiden, Leiden, Netherlands; 2) Department of Molecular Cell Biology and Immunology, VU University Medical Center, van den Boechorststraat 7, 1081 BT Amsterdam, The Netherlands,; 3) Kluyver Centre for Genomics of Industrial Fermentation, P.O box 5057, 2600 GA Delft, The Netherlands.

Galactofuranose-containing glycoconjugates are present in numerous microbes, many of which are pathogenic for humans. Metabolic aspects of the monosaccharide have proven difficult to elucidate, because galactofuranose metabolites and glycoconjugates are relatively unstable during analyses. Recent advances with genetic approaches have facilitated a better understanding of galactofuranose biosynthesis. Galactofuranose (Galf) the five-ring isomer of galactopyranose (Galp), is an essential component of the cell wall and required for a structural integrity [1-2]. Recently, it has been postulated that UDP-Galp, is converted to Galf by a UDP-galactopyranose mutase (UgmA) and subsequently transported into the Golgi by a putative UDP-Galf-transporter for the further biosynthesis of cell wall polymers such as galactomannan, galactoaminogalactan and cell wall glycoproteins (galactomanno-proteins) [3-4]. Based on homology searches, we have identified two putative UDP-Galf-transporters in A. niger. We have studied the function of the transporters by making deletions mutants (either single or double mutants) and by studying their localization by making GFP fusions. We conclude that the two putative UDP-Galf-transporters (named (UgtA and UgtB) have an overlapping function in UDP-Galf-transport and that both proteins are localized in Golgi equivalents. References: [1] Damveld, R.A. et al., 2008. Genetics 178 (2), 873-81; [2] Schmalhorst, P.S. et al. 2008, Euk. Cell 7 (8), 1268-77; [3] Engel, J. et al., 2009. J. Biol. Chem. 284; [4] Bernard, M., Latge, J. P., 2001. Med. Myc. 39, 9-17;.

104. Maltose permease-encoding mRNA is cleaved under induction condition of amylolytic gene expression in *Aspergillus oryzae*. Mizuki Tanaka, Takahiro Shintani, Katsuya Gomi. Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan.

Eukaryotic mRNA is degraded by two degradation pathways: the 5' to 3' degradation pathway by Xrn1 and the 3' to 5' degradation pathway by exosome-Ski complex. To investigate the mRNA degradation mechanism in filamentous fungi, we generated the disruptions of orthologous genes encoding mRNA degradation machinery in *Aspergillus oryzae*. Interestingly, the disruptants of *ski2* and *ski3*, which encode the components of Ski complex, showed the remarkable growth defect on minimal medium containing maltose or starch as a sole carbon source, whereas they normally grew on the medium with glucose or fructose as a sole carbon source. Northern blot analysis showed that the 3'-truncated fragment of mRNA encoding maltose permease (*malP*) was accumulated in Ski complex deficient mutants. Circularized RT-PCR analysis revealed that the *malP* mRNA was cleaved at a large stem-loop structure situated within the coding region. These results suggested that the *malP* mRNA is cleaved by endonuclease and the resultant 3'-truncated *malP* mRNA is degraded rapidly by 3' to 5' degradation pathway. In higher eukaryotes, it has been reported that the mRNAs encoding secreted and membrane proteins were cleaved by endoplasmic-reticulum (ER) endonuclease Ire1 during ER stress. Since *A. oryzae* produces copious amounts of amylolytic enzymes in the presence of maltose, we presumed that *malP* mRNA is cleaved by Ire1 with the induction of amylolytic gene expression. Therefore, we generated the double deficient mutant for Ski complex and AmyR, the regulator of amylolytic genes expression. The resultant double mutant showed normal growth on maltose medium, and 3'-truncated fragment of *malP* mRNA was not detected by Northern blot analysis. This result clearly indicated that *malP* mRNA is cleaved under induction condition of amylolytic gene expression in *A. oryzae*.

105. Functional characterisation of Rac GTPase in *Botrytis cinerea* reveals impact on polarity, cell cycle and pathogenicity. Anna Minz-Dub[1], Leonie Kokkelink[2], Paul Tudzynski[3], Amir Sharon[1]. 1) Department of Plant Sciences, Britannia 536, Tel-Aviv University, Tel-Aviv 69978; 2) Universität zu Köln, Biozentrum, Institut für Botanik, Zülpicher Str. 47 b, 50674 Koeln, Germany; 3) Institut für Biologie und Biotechnologie der Pflanzen ,Westfaelische Wilhelms-Universitaet Muenster, Schlossplatz 8, D-48143 Muenster, Germany.

Small GTPases of the Ras superfamily are involved in regulation of different cellular mechanisms including cell cycle and differentiation. Furthermore, small GTPase proteins are interconnected with many different signalling pathways. In this study we describe functional characterization of a Rho-type GTPase BcRac from the necrotrophic plant pathogen *Botrytis cinerea*. Role of this protein in cell cycle, development and pathogenicity is described. Expression of a constitutively active (CA) version of the BcRac protein, or deletion of the gene had a severe impact on fungal growth and differentiation. The mutant strains have polarity defects, they do not produce conidia, disease symptoms on plants are delayed, and they produce and accumulate increased amounts of ROS in culture. In addition, nuclear content and actin localization were altered in the CA-BcRac strain as compared to wild type. An effect of Rac-specific inhibitor NSC23766 on spore germination of wild type strain indicated that BcRac might be necessary for spore germination during G2/M phase. Based on our observations, BcRac is an important regulator of development in *B. cinerea*, and alteration of its activity disrupts the morphogenetic program and influences fungal infection.

106. Light matters: The transcription factor LTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. Julia Schumacher[1], Adeline Simon[2], Kim Cohrs[1], Muriel Viaud[2], Paul Tudzynski[1]. 1) IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany; 2) INRA, BIOGER, Avenue Lucien Brétignières, 78850 Grignon, France.

The lifecycle of *Botrytis cinerea/ Botryotinia fuckeliana* includes the formation of white mycelia generating pigmented conidiophores with macroconidia for propagation, pigmented sclerotia for over-wintering and sexual reproduction, microconidia for spermatization of the sclerotia, and the formation of apothecia as fruiting bodies on spermatized sclerotia. Full-spectrum light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed during incubation in constant darkness. The relevance of light for virulence of the fungus is not that clear, however, infections are observed under natural illumination conditions as well as in constant darkness. By a T-DNA insertional mutagenesis approach, we identified a novel virulence-related gene encoding a GATA-type transcription factor (TF) with homologues in *A. nidulans* (NsdD) and *N. crassa* (SUB-1). As transcription is induced by light (2.5-fold), it is called BcLTF1 for 'Light-regulated TF 1'. By deletion and over-expression of BcLTF1, we confirmed the predicted role of the TF in virulence, and discovered furthermore its extraordinary functions in regulating light-dependent differentiation processes (growth defect of Dbcltf1 in light, loss of sclerotia formation in darkness), the equilibrium between production and scavenging of reactive oxygen species (ROS), and secondary metabolism. Hence, microarray analyses (WT, Dbcltf1; dark vs. exposure to light for 1h) revealed that the expression levels of 206 out of 313 light-dependent genes are modulated by BcLTF1, including the genes of the putative carotenoid gene cluster and six out of eleven genes encoding TFs. In addition, the mutation of bcltf1 affects the expression of 1,616 genes irrespective of the light conditions, including the over-expression of known and so far uncharacterized secondary metabolism gene clusters. The over-expression of the gene encoding the alternative oxidase (AOX) and the under-expression of genes involved in oxidative stress responses are in accordance with the observed phenotypes of the deletion mutant, i.e. the hypersensitivity to exogenously applied oxidative stress even in the absence of light and the restoration of growth rates in continuous light by offering antioxidants, indicating that BcLTF1 is required to cope with oxidative stress that is caused by the exposure to light.

107. Functional analysis of genes in the mating type locus of *Botrytis cinerea*. Razak Bin Terhem, Joost Stassen, Jan van Kan. Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.

Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2, each containing two genes. Besides the archetypal genes encoding the MAT1-1-1 (alpha-domain) protein and the MAT1-2-1 (HMG-box) protein, each idiomorph contains one additional gene, designated *MAT1-1-5* and *MAT1-2-4*, respectively. Homologs of these genes are only found in closely related taxa, and their function is as yet unknown. Knockout mutants were generated in all four genes in the *B. cinerea* MAT locus, either in the MAT1-1 strain SAS56 or in the MAT1-2 strain SAS405. Mutants were crossed with a strain of the opposite mating type, either the wild type or a knockout mutant, in all possible combinations. Knockout mutants in the *MAT1-1-1* gene and the *MAT1-2-1* gene fail to show any sign of primordial outgrowth and are entirely sterile. This confirms the essential role of the alpha-domain protein and the HMG-box protein in the mating process. By contrast, mutants in the *MAT1-1-5* gene and the *MAT1-2-4* gene do produce stipes, but these fail to develop further into an apothecial disk. The *MAT1-1-5* and *MAT1-2-4* mutants show identical phenotypes, suggesting that these two genes jointly control the transition from stipe to disk development. RNA-seq data were obtained from a cross between two wild type strains and from a cross involving a *MAT1-1-5* knockout mutant, from tissue at the stage of transition from stipe to disk. Differential gene expression analysis was performed to identify genes that are possibly involved in development of the apothecial disk.

108. The role of hydrophobins in sexual development of *Botrytis cinerea*. Razak Bin Terhem[1], Matthias Hahn[2], Jan van Kan[1]. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 2) Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany.

Hydrophobins are small secreted proteins that play a role in a broad range of developmental processes in filamentous fungi, e.g. in the formation of aerial structures. Hydrophobins allow fungi to escape their aqueous environment and confer hydrophobicity to fungal surfaces. In *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana*), one class I and two class II hydrophobin genes have been identified, as well as a number of hydrophobin-like genes. Previous studies showed that hydrophobins are not required for conferring surface hydrophobicity to conidia and aerial hyphae. We investigated the role of hydrophobins in sclerotium and apothecium development. RNA seq analysis of gene expression during different stages of apothecium development revealed high expression of the *Bhp*1 (class I hydrophobin) gene and of the *Bhl*1 (hydrophobin-like) gene in certain stages, whereas *Bhp*2 and *Bhp*3 (class II hydrophobin) genes were always expressed at very low levels. We characterized different hydrophobin mutants: four single gene knockouts, three double knockouts as well as a triple knockout. Sclerotia of D*Bhp*1/D*Bhp*3 (double knock out) and D*Bhp*1/D*Bhp*2/D*Bhp*3 (triple knock out) mutants showed easily wettable phenotypes. These results indicate that hydrophobins Bhp1 and Bhp3 are important for normal development of sclerotia of *B. cinerea*. For analyzing apothecial development, a reciprocal crossing scheme was set up. Morphological aberrations were observed in crosses with some hydrophobin mutants. When the D*Bhp*1/D*Bhp*2 (double knock out) and D*Bhp*1/D*Bhp*2/D*Bhp*3 (triple knockout) mutants bearing a MAT1-1 mating type were used as maternal parents (sclerotia), and fertilized with microconidia of a wild type MAT1-2 strain, the resulting apothecia were swollen, dark brown in color and had a blotted surface. Instead of growing upwards, the apothecia in some cases fell down. This aberrant apothecial development was not observed in the reciprocal cross, when the same mutants were used as paternal parent (microconidia). These results indicate that the presence of hydrophobins Bhp1 and Bhp2 in maternal tissue is important for normal development of apothecia of *B. cinerea*.

109. The *pescadillo* homolog, controlled by Tor, coordinates proliferation and growth and response in *Candida albicans* yeast. Tahmeena Chowdhury[1], Niketa Jani[1], Folkert J. Van Werven[2], Robert J. Bastidas[3], Joseph Heitman[3], Julia R. Köhler[1]. 1) Division of Infectious Diseases, Boston Children's Hospital/Harvard Medical School, Boston, MA; 2) Institute for Integrative Cancer Research, MIT, Cambridge, MA; 3) Dept. of Genetics and Molecular Biology, Duke University, NC.

Candida albicans has evolved as a colonizer and opportunistic pathogen of mammals. Among fungi infecting humans, it is unique in the frequency with which it switches between growth as budding yeast and growth as pseudohyphal and hyphal filaments. In vitro and presumably in vivo, filaments constitutively produce yeast from their sub-apical compartments. This developmental step is required for dispersal of planktonic yeast from biofilms. The *C. albicans pescadillo* homolog PES1 is required for this lateral yeast growth. In eukaryotes, *pescadillo* homologs are involved in cell cycle progression and ribosome biogenesis, processes that respond to nutrient availability. This work investigated the potential role of *C. albicans* PES1 in the Tor signaling pathway, which is a major nutrient signaling cascade. Results show that Tor signaling controls Pes1 expression and localization. *C. albicans* yeast but not hyphae require Pes1 for proliferation, and for proliferation arrest upon Tor1 inhibition with rapamycin. Pes1 inactivation via a temperature-sensitive allele leads to defective exit of starved cells from the cell cycle. Pes1 inactivation also leads to rapid loss of phosphorylation of ribosomal protein S6, a marker of translational activity, as does Tor1 inhibition and genetic perturbation of Tor1 activation. These data support a role for Pes1 downstream of Tor1 in coordinating cell cycle progression with protein synthesis. As all cells must coordinate proliferation and growth, investigating why the requirement for Pes1 in this role is yeast-specific will inform our understanding of morphogenesis and Tor signaling in *C. albicans*.

110. Uncovering the mechanisms of thermal adaptation in *Candida albicans*. Michelle Leach[1,2], Susan Budge[2], Louise Walker[2], Carol Munro[2], Alistair Brown[2], Leah Cowen[1]. 1) Department of Molecular Genetics, University of Toronto, Medical Sciences Building, 1 Kings College Circle, Toronto, Ontario, Canada, M5S 1A8; 2) Aberdeen Fungal Group, School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, UK.

The heat shock response is governed by one of the most highly conserved networks in eukaryotic cells. Upon sensing a sudden temperature upshift, the heat shock transcription factor (Hsf1) is rapidly phosphorylated and activated, leading to the induction of numerous genes that mediate thermal adaptation, including heat shock genes that encode molecular chaperones. We have shown that the major fungal pathogen of humans, *Candida albicans*, has retained a bona fide heat shock response even though it is obligatorily associated with warm-blooded animals [*Molec. Micro*. 74, 844]. Furthermore, this thermal adaptation is essential for the virulence of *C. albicans* [*Fungal Gen. Biol*. 48, 297]. To identify signalling pathways that contribute to long-term thermal adaptation resistance in *C. albicans* we performed unbiased genetic screens for protein kinase mutants that display temperature sensitivity. This screen reproducibly highlighted several key signalling pathways associated with cell wall remodelling: the Hog1, Mkc1 and Cek1 pathways. None of these pathways are essential for Hsf1 phosphorylation and activation; each pathway contributing to heat shock adaptation independently of Hsf1. We demonstrate that these pathways are differentially activated during heat shock, and that there is crosstalk between these pathways, with high temperatures contributing to increased resistance to cell wall stress in the long term, and oxidative stress in the short term. Critically, this crosstalk between thermotolerance and other types of stress adaptation is mediated by the molecular chaperone Hsp90, whose down-regulation reduces the resistance of *C. albicans* to proteotoxic stresses. Hsp90 depletion also affects cell wall biogenesis by impairing activation of these signalling pathways. Furthermore, we show that Hsp90 interacts with and down-regulates Hsf1 thereby modulating short-term thermal adaptation. Therefore, Hsp90 lies at the heart of heat shock adaptation, modulating the short-term Hsf1-mediated activation of the classic heat shock response, coordinating this response with long term thermal adaptation via Mkc1- Hog1- and Cek1-mediated cell wall remodelling.

111. Characterisation of contact-dependant tip re-orientation in *Candida albicans* hyphae. Darren Thomson, Silvia Wehmeier, Alex Brand. Aberdeen Fungal Group, Aberdeen University, Aberdeen, United Kingdom.

Candida albicans is a pleiomorphic fungus that lives as a commensal yeast in the human body but can become pathogenic in susceptible patient groups. Virulence is strongly linked with the production of penetrative hyphae that can adhere to and invade a wide range of substrates, including blood vessels, organ tissue, keratinised finger-nails and even soft medical plastics. Using live-cell imaging and nanofabricated surfaces, we are characterising the spatio-temporal dynamics of contact-induced hyphal tip behaviour (thigmotropism). To test whether tip re-orientation responses positively correlate with levels of hyphal adhesion, we generated substrates with increasing adhesive force. Hyphal tip re-orientation was absent in poorly-immobilised hyphae and a threshold adhesive force was required sub-apically to generate the hyphal tip pressure required for re-orientation. Interestingly, sub-threshold adhesion resulted in sub-apical hyphal bending. Localization of fluorescent protein markers for the Spitzenkörper and the Polarisome (Mlc1-YFP and Spa2-YFP, respectively) showed that *C. albicans* hyphal tips grow in an asymmetric, ‘nose-down’ manner on a surface. Additionally, hyphal tips can detect surface stiffness and show a distinct preference for nose-down growth on the softer of two substrates. Localisation of fluorescent cell-cycle reporter proteins over time revealed that hyphal tip contact slowed the cell-cycle, suggesting that tip-contact perturbs cell-cycle mechanics. Finally, we examined the role of cytoskeleton regulators in thigmotropism and determined the force that can be generated by the hyphal tip. Our results suggest that *C. albicans* hyphae

can exert sufficient force to penetrate human epithelial tissue without the need for secreted enzyme activity. This is consistent with the observed hyphal penetration of medical-grade silicone, which has a similar Young's modulus to human cartilage.

112. Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase. Audrey M.V. Ah-Fong, Howard S. Judelson. Plant Pathology & Microbiology, University of California, Riverside, CA.

The dual-specificity phosphatase Cdc14 is best known as a regulator of cell cycle events such as mitosis and cytokinesis in yeast and animal cells. However, the diversity of processes affected by Cdc14 in different eukaryotes raises the question of whether its cell cycle functions are truly conserved between species. Analyzing Cdc14 in *Phytophthora infestans* should provide further insight into the role of Cdc14 since this organism does not exhibit a classical cell cycle. Prior study in this organism already revealed novel features of its Cdc14. For example, instead of being post-translationally regulated like its fungal and metazoan relatives, PiCdc14 appears to be mainly under transcriptional control. It is absent in vegetative hyphae where mitosis occurs and expressed only during the spore stages of the life cycle which are mitotically quiescent, in contrast to other systems where it is expressed constitutively. Since transformants overexpressing PiCdc14 exhibit normal nuclear behavior, the protein likely does not play a critical role in mitotic progression although PiCdc14 is known to complement a yeast Cdc14 mutation that normally arrests mitosis. Further investigation into the role of PiCdc14 uncovered a novel role. Subcellular localization studies based on fusions with fluorescent tags showed that PiCdc14 first appeared in nuclei during early sporulation. During the development of biflagellated zoospores from sporangia, PiCdc14 transits to basal bodies, which are the sites from which flagella develop. A connection between Cdc14 and flagella is also supported by their phylogenetic distribution, suggesting an ancestral role of Cdc14 in basal bodies and/or flagellated cells. To help unravel the link between PiCdc14 and the flagella apparatus, searches for its interacting partners using both yeast two hybrid and affinity purification are underway. Together with colocalization studies involving known basal body/centrosome markers such as centrin and gamma-tubulin, the location and hence the likely roles of PiCdc14 will be revealed.

113. *Colletotrichum orbiculare* Bub2-Bfa1 complex, a spindle position checkpoint (SPOC) component in *Saccharomyces cerevisiae*, is involved in proper progression of cell cycle. Fumi Fukada[1], Ayumu Sakaguchi[2], Yasuyuki Kubo[1]. 1) Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan.

Colletotrichum orbiculare is an ascomycete fungus that causes anthracnose of cucumber. In *Saccharomyces cerevisiae*, the orientation of the mitotic spindle with respect to the polarity axis is crucial for the accuracy of asymmetric cell division. A surveillance mechanism named spindle position checkpoint (SPOC) prevents exit from mitosis when the mitotic spindle fails to align along the mother-daughter polarity axis. *BUB2* is a component of SPOC and constitutes the main switch for the mitotic exit network (MEN) signaling. We identified and named this homolog as *CoBUB2* in *C. orbiculare* and generated gene knock-out mutants. First, we observed morphogenesis and pathogenesis of the *cobub2* mutants. The *cobub2* mutants formed abnormal appressoria and penetration hyphae on model substrates, and the *cobub2* mutants also showed attenuate pathogenesis to cucumber leaves. Second, we observed mitosis based on mitotic spindle behavior and nuclear DAPI staining during appressorium development. In the wild type, mitosis occurred in appressorium developing conidia after 4h incubation, whereas interestingly, in the *cobub2* mutants, mitosis occurred in pre-germinated conidia after 2h incubation. After development of appressorium, in some germlings the daughter nucleus was delivered from conidia to appressoria, and the others perform second round of mitosis in appressorium developing conidia after 4h incubation. Third, we evaluated the timing of S phase and M phase during appressorium development in wild type and the *cobub2* mutants by cell cycle specific inhibitors. In the *cobub2* mutants, it was shown that the transition period from G1 phase to S phase accelerated about 2h than that of the wild type. Last, in *S. cerevisiae*, Bub2 forms GTPase activating protein (GAP) complex with Bfa1, and Bub2-Bfa1 GAP complex constitutes SPOC. Then we named homolog of *BFA1* as *CoBFA1* in *C. orbiculare* and generated *cobfa1* mutants. From observation of nuclear division, the *cobfa1* mutants showed similar behavior of nuclear division to the *cobub2* mutants. Therefore, it is assumed that CoBub2 forms GAP complex with CoBfa1, however, CoBub2-CoBfa1 GAP complex has different function from that in *S. cerevisiae* maintaining G1 phase duration or setting up the proper time of S phase.

114. Metazoan-like mitotic events in the basidiomycetous budding yeast *Cryptococcus neoformans* - a human fungal pathogen. L. Kozubowski[1,2], V. Yadav[3], G. Chatterjee[3], M. Yamaguchi[5], I. Bose[4], J. Heitman[2], K. Sanyal[3]. 1) Department of Medicine, Division of Infectious Diseases, Duke University Medical Center, Durham, NC, USA; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 3) Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 4) Department of Biology, Western Carolina University, Cullowhee, NC, USA; 5) Medical Mycology Research Center, Chiba University, Chiba, Japan.

Mitosis in ascomycetous budding yeasts is characterized by several features that are distinct from those of metazoans. In *Saccharomyces cerevisiae*, centromeres are always clustered in a single spot, the kinetochores are fully assembled for the majority of the cell cycle, and the nuclear envelope (NE) does not break down (closed mitosis). Currently it is not clear how these mechanisms evolved or whether these features are a universal characteristic hallmark of the budding mode of cellular division. Here we report an analysis of key mitotic events in the basidiomycetous human fungal pathogen *Cryptococcus neoformans*. The dynamics of microtubules, the kinetochore, NE and the nucleolus were analyzed by time-lapse microscopy using fluorescently tagged proteins. In striking contrast to ascomycetous budding yeast, centromeres in *C. neoformans* were not clustered in non-dividing cells. Prior to mitosis, centromeres underwent gradual clustering, eventually forming a single spot, which then migrated into the daughter cell where the chromosomal division occurred. One set of chromosomes migrated back to the mother cell and subsequent de-clustering of centromeres occurred in both cells. Analysis of individual components of the kinetochore indicated that kinetochores assemble in a step-wise manner in *C. neoformans*. While the inner kinetochore (Cse4, Mif2) was present throughout the entire cell cycle, the middle kinetochore (Mtw1) assembled prior to mitosis when centromeres underwent clustering, and this was then followed by assembly of the outer kinetochore (Dad1, Dad2). Formation of the outer kinetochore during mitosis, as observed in metaozoans that undergo an open mitosis, prompted us to examine the fate of the NE at various cell cycle stages. Several lines of evidence suggested that *C. neoformans* undergoes a semi-open mitosis. The nuclear pore marker GFP-Nup107, and a nucleolar marker GFP-Nop1 dispersed into the cytoplasm during metaphase, a nuclear membrane marker Ndc1 exhibited a localization pattern that also suggests a partial opening of the NE during mitosis. A semi-open mitosis was further confirmed by transmission electron microscopy. In summary, our data demonstrate that key mitotic events in *C. neoformans* are similar to that of metazoan cells. This study sheds new light on the evolution of mitosis during fungal speciation.

115. Distinctive Mitotic Localization of a Novel Suppressor of *nimA1* Provides New Insight into NIMA Function. Jennifer R. Larson, Stephen A. Osmani. Department of Molecular Genetics, The Ohio State University, Columbus, OH.

The NIMA kinase is an essential regulator of mitotic events in *Aspergillus nidulans*. Not only is NIMA essential for initiating mitosis its overexpression can prematurely induce mitotic events including DNA condensation and nuclear pore complex (NPC) disassembly in *A. nidulans* and human cells. One of the key roles for NIMA at the onset of mitosis is its regulation of NPCs. A previous study aimed at identifying suppressors of the temperature-sensitive *nimA1*

allele isolated two NPC proteins, which were named SONA and SONB for Suppressors Of *NimA1*. Although NIMA is essential for mitotic entry there is also evidence that NIMA and conserved related kinases have functions later in mitosis and in the DNA damage response. To further characterize the roles of NIMA we designed a genetic screen to isolate additional suppressors of *nimA1* that also cause conditional temperature-dependent DNA damage sensitivity. Our expectation was the identification of additional genes involved in NIMA regulation and in the DNA damage response. Here we describe one such gene, which we have named *sonC*. SonC contains a unique Zn(II)Cys6 binuclear DNA binding domain, which is highly conserved among the Ascomycota. Deletion of *sonC* results in swollen, ungerminated spores, suggesting it is essential for a core growth process. As expected for a DNA binding protein, SonC localizes to nuclei during interphase. Interestingly, dual fluorescence imaging of SonC with histone H1 during mitosis revealed that a portion of SonC localizes with histone H1 along a distinct projection of chromatin that juts away from the main, condensed chromatin mass, which we hypothesize may be the NOR. Supporting this hypothesis, the region of DNA that likely forms the projection is cradled by the nucleolus prior to mitosis as seen by colocalization studies of SonC with the nucleolar protein Bop1. As mitosis proceeds, the H1 histones are evicted from the middle region of this projection but not at its distal end. This indicates that the chromatin in this region of the genome is altered during mitotic progression and we are testing the idea that SonC might be important for NOR condensation and/or nucleolar disassembly during its mitotic segregation. Because SonC was identified as a suppressor of NIMA we propose that NIMA may have a function in regulating nucleolar disassembly during mitosis.

116. Investigating Cell Cycle-Regulated Control of Appressorium Morphogenesis in the Rice Blast Fungus *Magnaporthe oryzae*. Wasin Sakulkoo, Nicholas J. Talbot. School of Biosciences, University of Exeter, Exeter EX4 4QD, United Kingdom.

The rice blast fungus *Magnaporthe oryzae* elaborates specialized infection structures called appressoria to gain entry into rice plant tissue. The initiation of appresssorium morphogenesis has previously been shown to require a single round of mitosis in the germ tube, shortly after spore germination. On daughter nucleus migrates to the incipient appressorium at the germ tube tip and the other daughter nucleus moves back to the conidial cell from which the germ tube originates. We reasoned that an S-phase checkpoint mediates the apical-isotropic switch leading to swelling of the germ tube tip. Perturbation of DNA synthesis by hydroxyurea (HU) blocks the initiation of appressorium formation, but only when applied within 3-4h of spore germination, prior to S-phase. Here, we report investigations regarding the interplay between cell cycle control and operation of the Pmk1 Mitogen-activated protein kinase cascade, which is essential for appressorium morphogenesis in *M. oryzae*. Furthermore we report changes in the global pattern of gene expression of HU-treated conidia which has been carried out in order to determine the identity of morphogenetic genes that are controlled by the S-phase checkpoint. Progress on understanding the genetic control of early appressorium development will be presented.

117. THE ROLE AND TRAFFIC OF CHITIN SYNTHASES IN *Neurospora crassa*. R. Fajardo[1], R. Roberson[2], B. Jöhnk[3], Ö. Bayram[3], G.H. Braus[3], M. Riquelme[1]. 1) Department of Microbiology, CICESE, Ensenada, Mexico; 2) School of Life Sciences, Arizona State University, Arizona, USA; 3) Molecular Microbiology and Genetics, Georg-August University, Göttingen, Germany.

Chitin is one of the most important carbohydrates in the cell wall in filamentous fungi. Chitin synthases (CHS) are involved in the addition of N-acetylglucosamine monomers to form chitin microfibrils. The filamentous fungus *Neurospora crassa* has one representative for each of the seven CHS classes described. Previous studies have shown that in *N. crassa*, CHS-1, CHS-3 and CHS-6, are concentrated at the core of the Spitzenkörper and in forming septa and seem to be transported in different populations of chitosomes. In this study we have endogenously tagged *chs-2*, *chs-4*, *chs-5* and *chs-7* with *gfp* to study their distribution in living hyphae of *N. crassa*. CHS-5 and CHS-7 both have a myosin motor-like domain at their amino termini, suggesting that they interact with the actin cytoskeleton. CHS-2 and CHS-7, appeared solely involved in septum formation. As the septum ring developed, CHS-2-GFP moved centripetally until it localized exclusively around the septal pore. CHS-4 and CHS-5 were localized both at nascent septa and in the core of the Spk. We observed a partial colocalization of CHS-1-mCherry and CHS-5-GFP in the Spk. Total internal reflection fluorescence microscopy (TIRFM) analysis revealed putative chitosomes containing CHS-5-GFP moving along wavy tracks, presumably actin cables. Collectively our results suggest that there are different populations of chitosomes, each containing a class of CHS. Mutants with single gene deletions of *chs-1*, *chs-3*, *chs-5*, *chs-6*, or *chs-7* grew slightly slower than the control strain (FGSC#9718 and FGSC#988); only *chs-6D* displayed a marked reduction in growth. Both *chs-5D* and *chs-7D* strains produced less aerial hyphae and conidia. The double mutant *chs-5D; chs-7D* showed less growth, aerial hyphae production and conidiation than the single mutant *chs-5D*, but not than the *chs-7D* single mutant. A synergic effect was observed in double mutant *chs-1D; chs-3D*, in which growth, aerial hyphae production and conidiation were significantly decreased. During the sexual cycle, after homozygous crosses, *chs-3D* and *chs-7D* strains did not produce perithecia and *chs-5D* produced less perithecia. We are analyzing chitin and glucan synthase activities in these single and double mutants. Additionally, we are conducting pulldown assays, and mass spectrometry to identify putative proteins that are interacting with CHS.

118. DFG5 and DCW1 cross-link Cell Wall Proteins into the Cell Wall Matrix. Abhiram Maddi, Jie Ao, Stephen J. Free. Dept Biological Sci, SUNY Univ, Buffalo, Buffalo, NY.

The cell wall is an essential organelle for the growth and survival of a fungus. The cell wall structure consists of matrix of cross-linked chitin, glucans, and cell wall glycoproteins. In Neurospora crassa, we have shown that the DFG5 and DCW1 proteins function in cross-linking the cell wall proteins into the cell wall matrix. We have also shown that the Candida albicans DFG5 and DCW1 proteins are required for the cross-linking of cell wall proteins into the cell wall. The DFG5 and DCW1 proteins are predicted to have a-1,6-mannanase activity. Our results suggest that they function in transglycosylation reactions between a-1,6-mannans, which are found in galactomannan and the outer chain mannan structures present as modifications on cell wall proteins, and cell wall glucans. These galactomannans and outer chain mannans are modifications to the N-linked oligosaccharides attached to cell wall glycoproteins. As a result of these transglycosylation reactions, the cell wall proteins are effectively cross-linked into the cell wall. The DFG5 and DCW1 enzymes are excellent targets for the development of anti-fungal agents that could disrupt cell wall biosynthesis.

119. Cell wall biology to illuminate mechanisms of pathogenicity in *Phytophthora infestans*. Laura Grenville-Briggs, Stefan Klinter, Francisco Vilaplana, Annie Inman, Hugo Mélida, Osei Ampomah, Vincent Bulone. Division of Glycoscience, Royal Institute of Technology, (KTH), Stockholm, Sweden.

The cell wall is a dynamic extracellular compartment protecting the cell, providing rigidity, and playing an essential role in the uptake of molecules and signalling. In pathogenic organisms, the cell wall is at the forefront of disease, providing contact between the pathogen and host. Using a multidisciplinary approach, we seek to understand the role of the cell wall in oomycete disease, both as a communication centre with the host organism and as a compartment that is continually reshaped and strengthened throughout the lifecycle, to penetrate and colonise the host. Understanding these mechanisms in more detail will pave the way for better control of oomycete diseases. We are combining novel chemical genomics approaches with state-of-the-art biochemistry and biophysics to study the cell wall and to develop new anti-oomycete drugs. *P. infestans* produces a variety of spores and infection structures that are essential for disease development throughout its lifecycle. In particular thick-walled sporangia release wall-less motile zoospores that rapidly synthesise a cell wall upon contact with host plant cells. These cysts further differentiates to produce appressoria which build up

turgor pressure and act as a focal point for cell wall degrading enzymes to penetrate the host cell. A highly strengthened cell wall is thus essential for the onset of infection. Here we present the results of our detailed biochemical analyses, using GC-MS and methylation analysis to determine the neutral sugar composition and glycosidic linkages of the cell wall structural carbohydrates present at these key points in the lifecycle. Having previously established an essential role for a cellulosic cell wall in appressorium production and infection of potato by *P. infestans* (Grenville-Briggs et al 2008), we are now working to elucidate the precise functions of the individual cellulose synthase (*CesA*) genes. Silencing each *CesA* using RNAi reveals overlapping functions with subtle differences in phenotype. These results will be presented. Since the genome of *P. infestans* also contains a putative chitin synthase, but hyphal cell walls are devoid of measurable chitin we are also investigating the role of this gene in the *P. infestans* cell wall and in pathogenicity and here we present the latest findings of this work.

120. Analysis of the cell wall integrity (CWI) pathway in *Ashbya gossypii*. Klaus B Lengeler, Lisa Wasserström, Andrea Walther, Jürgen Wendland. Carlsberg Laboratory, Yeast Biology, DK-1799 Copenhagen V, Denmark.

Fungal cells are constantly exposed to rapidly changing environmental conditions, in particular considering their osmotic potential. The cell wall takes on an important function in protecting the fungal cell from external stresses and controlling intracellular osmolarity, but it is also required to maintain regular cell shape. At the same time, cells must still be able to remodel the rigid structure of the cell wall to guarantee cell expansion during cell differentiation processes. While several signaling pathways contribute to the maintenance of the cell wall, it is the cell wall integrity (CWI) pathway that is most important in regulating the remodeling of the cell wall structure during vegetative growth, morphogenesis or in response to external stresses. To characterize the CWI pathway in the filamentous ascomycete *Ashbya gossypii* we generated deletion mutants of several genes encoding for the most important components of the CWI pathway including potential cell surface sensors (e.g. *AgWSC1*), the following downstream protein kinases including a MAPK signaling module (*AgPKC1*, *AgBCK1*, *AgMKK1* and *AgMPK1*), and transcription factors known to be involved in CWI signaling (e.g. *AgRLM1*). An initial characterization of the corresponding mutants is presented. While a mutant in *Agpkc1* shows a strong general growth defect, mutants in several other components of the CWI pathway, in particular in the MAPK module, show a noticeable colony lysis phenotype. Finally, we show that the colony lysis phenotype may be useful to easily isolate recombinant proteins from *A. gossypii*.

121. Dynamics of exocytic markers and cell wall alterations in an endocytosis mutant of *Neurospora crassa*. Rosa R. Mouriño-Pérez, Ramón O. Echauri-Espinosa, Arianne Ramírez-del Villar, Salomón Bartnicki-García. Microbiology Department, CICESE, Ensenada, B.C., Mexico.

Morphogenesis in filamentous fungi depends principally on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. Previously, we found that coronin deletion (*Neurospora crassa* mutant, D*crn-1*) causes a decrease in endocytosis (measured by the rate of uptake of FM4-64) together with marked alterations in normal hyphal growth and morphogenesis accompanied by irregularities in cell wall thickness. The absence of coronin destabilizes the cytoskeleton and leads to interspersed periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. By confocal microscopy, we found that CRIB-GFP was present In wild-type hyphae as a thin hemispherical cap under the apical dome, i. e. when growing in a polarized fashion and with regular hyphoid morphology. In the D*crn-1* mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the D*crn-1* mutant correlated with the morphological irregularity of the hyphae. We found that the D*crn-1* mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the D*crn-1* mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

122. Comprehensive genome-based analysis of cell wall biosynthesis in the filamentous phytopathogen *Ashbya gossypii*. R. Capaul[1], M. Finlayson[1], S. Voegeli[1], A. I. Martinez[2], Q. Y. Yin[3], C. de Koster[3], F. M. Klis[3], P. Philippsen[1], P. W. J. de Groot[2]. 1) Biozentrum, Molecular Microbiology, University of Basel, Klingelbergstr. 50-70, CH 4056 Basel, Switzerland; 2) Regional Center for Biomedical Research, Albacete Science & Technology Park, University of Castilla-La Mancha, Spain; 3) Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

The filamentous ascomycete Ashbya gossypii and the yeast Saccharomyces cerevisiae are phylogenetically closely related. It is not known how A. gossypii has evolved an exclusively hyphal growth mode with very rapid apical extension requiring cell wall expansion rates that are up to 40-fold faster compared to S. cerevisiae. The genome of A. gossypii encodes 44 putative cell wall-associated GPI proteins, 10 without a homolog in S. cerevisiae. This analysis also revealed amplification of several cell wall protein-encoding genes, notably CWP1. Transcriptome studies showed that one third of the CWP-encoding genes are expressed at higher levels than ribosomal protein genes. Mass spectrometric analysis of protein extracts from purified walls of rapidly growing hyphae resulted in the identification of 14 covalently bound cell wall proteins (CWPs). Some CWPs that are common in hemiascomycetes are missing in A. gossypii. On the other hand, the chitin deacetylase Cda1/Cda2 was identified in addition to three novel proteins (Agp1, Awp1, and Sod6), all without homologs in baker's yeast (NOHBYs). Phenotypic analysis confirmed the importance of these NOHBYs for cell wall integrity. Interestingly, hyphal walls of A. gossypii contain very little chitin and orthologs of genes required for cell wall remodeling and degradation of septa during cell division in S. cerevisiae show low expression or are absent. Conclusions: Loss of distinct cell wall genes, acquisition of novel genes, and amplification as well as increased expression of evolutionary conserved fungal cell wall genes led to the evolution of fast polar surface expansion of A. gossypii hyphae.

123. cAMP regulation in *Neurospora crassa* conidiation. Wilhelm Hansberg, Sammy Gutiérrez, Itzel Vargas, Miguel-Ángel Sarabia, Pablo Rangel. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México D.F., México.

In *N. crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development [1]. Each transition is started by an unstable hyperoxidant state (HO) and results in growth arrest, autophagy, antioxidant response and an insulation process from dioxygen [2,3]. These responses stabilize the system and growth can restart in the differentiated state. We found that *ras-1*^{bd} has increased ROS formation during conidiation resulting in increased aerial mycelium growth and increased submerged conidiation. Different *ras-1* point mutations were generated that affected growth and conidiation. Only three proteins have a predicted RAS association domain: NRC-1, the STE50p orthologue (STE50) and adenylate cyclase (AC). The D*ncr-1* was more resistant whereas the D*ste50* more sensitive to added H_2O_2. The AC mutant strain *cr-1* affects vegetative growth and aerial hyphae formation. Oxidative stress and RAS-1 determined partially cAMP levels during the first two HOs of the conidiation process. Higher cAMP levels than Wt were observed in *ras-1*^{bd}. In both strains, [cAMP] decreased within minutes at the start of the first two HOs and thereafter, as rapidly, levels recover to initial values. *N. crassa* has a high

(PDE_H) and a low affinity (PDE_L) phosphodiesterases. The D*pde*$_H$ strain grows slow and does not conidiate; no evident phenotype was reported for D*pde*$_L$. We found that PDE_L was mainly responsible for the cAMP decrease during the first HO and that hyphal adhesion was retarded in D*pde*$_L$. Both PDE_H and PDE_L were responsible for cAMP decrease during the second HO. H_2O_2 and low Ca^{++} activated PDE_L and inhibited PDE_H. This opposite regulation can explain the cAMP decrease during the HOs of the *N. crassa* conidiation process. [1] Toledo I *et al.* (1986) Aerial growth in Neurospora crassa: characterization of an experimental model system. Exp Mycol. **10**:114-125. [2] Hansberg W; Aguirre J (1990) Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J Theoret Biol **142**:201-221. [3] Hansberg W *et al.* (2008) Cell differentiation as a response to oxidative stress. In: Stress in Yeasts & Filamentous Fungi (Ed. Avery *et al.*) Elsevier IBSN 978-0-12-374184-4.

124. Ste12 is a negative regulator of conidiation and cell wall lytic enzymes production in response to nitrogen deprivation and light in Trichoderma atroviride. Maria Fernanda Nieto-Jacobo[1], Alfredo Herrera-Estrella[2], Alison Stewart[1], Artemio Mendoza-Mendoza[1]. 1) Bioprotection Research Centre, Lincoln University, Lincoln, Canterbury, New Zealand; 2) Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del IPN Sede Irapuato, Irapuato 36821, Guanajuato, Mexico.

Ste12 is a transcription factor found exclusively in the fungal kingdom. In *Saccharomyces cerevisiae*, Ste12 regulates mating and invasive/pseudohyphal growth, while in saprophytic and parasitic filamentous fungi Ste12-like proteins control mating, plant penetration and invasive growth. Ste12 and Ste12-like proteins are downstream components of the MAPK PMK1 pathway which are capable of regulating several genes encoding fungal virulence factors involved in both plant and animal infection. Among the virulence factors are diverse range of lytic enzymes and cell surface components. Several members *Trichoderma* genus are mycoparasites of plant fungal pathogens; so they are widely used as biocontrol agents. In addition, *Trichoderma* spp. penetrate plant roots and establish beneficial relationships with their host. One crucial element in biocontrol activity and root colonization of *Trichoderma* is the synthesis of lytic enzymes. Several lytic enzymes in *Trichoderma* are regulated by nitrogen metabolite repression. Here we observed that the *ste12-like* transcription factor gene is highly up-regulated when *Trichoderma* is grown on nitrogen depleted medium. To find the role of ste12 in *Trichoderma*, a *ste12-like* orthologue gene was deleted in *T. atroviride* and the effects on fungal development and response to different biotic and abiotic stimuli evaluated. Our results demonstrate that growth and conidiation of a *T. atroviride* Ste12-like mutant was only slightly altered in complete media. We evaluated the ability of the Dste12-like mutant to use a variety of nitrogen sources using Biolog microtiter plates. We noticed that when essential amino acids are used as the sole nitrogen source, the deletion mutant grew faster than the wild type, however this situation did not occur when the same amino acids were used as the sole carbon source. In addition, induction of conidiation in response to light or mechanical injury was stronger in the Dste12-like mutants than in the wild type but only when a secondary nitrogen source was used in the medium. Finally we observed that some lytic enzymes are differently produced between the wild type and Dste12-like mutants under nitrogen deprivation conditions. We propose that the *T. atroviride* Ste12-like orthologue regulates lytic enzymes and conidiation by a mechanism that involves nitrogen catabolite repression.

125. Black holes in fungal virulence: loss of RNAi in C. gattii outbreak strains reveals a novel RNAi factor. Marianna Feretzaki, Xuying Wang, Blake Bilmyre, Joseph Heitman. Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Genome instability and mutations provoked by transposon movement are counteracted by novel defense mechanisms in organisms as diverse as fungi, plants, and mammals. In the human fungal pathogen *Cryptococcus neoformans* we have previously characterized an RNAi silencing pathway that defends the genome against mobile elements and artificially introduced repeats of homologous DNA. Repetitive transgenes and transposons are silenced by an RNAi-dependent pathway during sexual development (sex-induced silencing, SIS) and during vegetative mitotic growth (MIS). RNAi silencing pathways are conserved in the *Cryptococcus* pathogenic species complex and are mediated by core RNAi components, including an RNA-dependent RNA polymerase (Rdp1), Argonaute (Ago1) and Dicer (Dcr1 and Dcr2). Surprisingly, all of the canonical known RNAi components are missing from all *C. gattii* VGII strains, the molecular type responsible for the North American Pacific Northwest outbreak. To identify novel components of the RNAi pathway, we surveyed the genome of the *C. gattii* R265 isolate for missing genes. One of the most interesting is *ZNF3*. In previous studies we found that Znf3, a protein with three zinc finger domains, is required for opposite- and same-sex mating in *C. neoformans* var. *neoformans*. Surprisingly, in *C. neoformans* var. *grubii ZNF3* is not essential for sexual development. However, it is required for mitotic- and sex-induced silencing via RNAi. SIS is less efficient in *znf3*D unilateral matings and is abolished in *znf3*D x *znf3*D bilateral matings, similar to the phenotypes of *rdp1*D mutants. Znf3 is also required for transgene-induced mitotic silencing; *znf3*D mutations abrogate silencing of repetitive transgenes during vegetative growth. Znf3 tagged with mCherry is localized in the cytoplasm in bright, distinct foci. Co-localization of Znf3 with the P-body marker Dcp1-GFP further supports the hypothesis that Znf3 is a novel element of the RNAi pathway and operates to defend the genome during sexual development and vegetative growth.

126. The Crz1/Sp1 transcription factor of Cryptococcus neoformans is activated by calcineurin and regulates cell wall integrity. Sophie Lev[1], Desmarini Desmarini[1], Methee Chayakulkeeree[2], Tania Sorrell[1], Julianne Djordjevic[1]. 1) Centre for Infectious Diseases and Microbiology, Sydney Medical School and Westmead Millennium Institute, University of Sydney, Westmead 2145 NSW, Australia; 2) Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Cryptococcus neoformans survives host temperature and regulates cell wall integrity via a calcium-dependent phosphatase, calcineurin. However, downstream effectors of *C. neoformans* calcineurin are largely unknown. In *S. cerevisiae* and other fungal species, a calcineurin-dependent transcription factor Crz1 translocates to nuclei upon activation and triggers expression of target genes. We now show that the *C. neoformans* Crz1 ortholog (Crz1/Sp1), previously identified as a protein kinase C target during starvation, is a *bona fide* target of calcineurin under non-starvation conditions, during cell wall stress and growth at high temperature. Both the calcineurin-defective mutant, *Dcna1*, and a *CRZ1/SP1* mutant (*Dcrz1*) were susceptible to cell wall perturbing agents. Furthermore, expression of the chitin synthase encoding gene, *CHS6*, was reduced in both mutants. We tracked the subcellular localization of Crz1-GFP in WT *C. neoformans* and *Dcna1* in response to different stimuli, in the presence and absence of the calcineurin inhibitor, FK506. Exposure to elevated temperature (30-37°C vs 25°C) and extracellular calcium caused calcineurin-dependent nuclear accumulation of Crz1-GFP. Unexpectedly, 1M salt and heat shock triggered calcineurin-independent Crz1-GFP sequestration within cytosolic and nuclear puncta. To our knowledge, punctate cytosolic distribution, as opposed to nuclear targeting, is a unique feature of *C. neoformans* Crz1. We conclude that Crz1 is selectively activated by calcium/calcineurin-dependent and independent signals depending on the environmental conditions.

127. A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrinal Signaling. Lingi Wang, Xunyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Microbes live mostly in a social community rather than in a planktonic state. Such communities have complex spatiotemporal patterns that require intercellular communication to coordinate gene expression. Here, we demonstrate that *Cryptococcus neoformans*, a model eukaryotic pathogen, responds to an extracellular signal in constructing its colony morphology. The signal that directs this community behavior is not a molecule of low molecular weight

like pheromones or quorum sensing molecules, but a secreted protein. We successfully identified this protein as the conserved adhesin Cfl1 in the extracellular matrix. The released Cfl1 acts as an auto-induction signal to stimulate neighboring cells to phenocopy Cfl1-expressing cells. We propose that such adhesin/matrix-initiated communication system exists in divergent microbes and our work represents the first adhesin/matrix-mediated signaling mechanism in simple eukaryotes.

128. The PacC Signal Transduction Pathway regulates Sexual Development in Neurospora crassa. Chinnici Jennifer, Arnold Jason, Stephen J. Free. Dept Biological Sci, SUNY Univ, Buffalo, Buffalo, NY.

As is common in the ascomycetes, the Neurospora crassa life cycle has both asexual and sexual developmental phases. Sexual development in N. crassa is characterized by the formation of a protoperithecium, fertilization, and the maturation of the protoperithecium to form a perithecium. In a screening experiment, we identified over 600 isolates from the N. crassa single gene deletion library that are unable to complete sexual development. Many of these are affected in the process of anastomosis, and we have previously reported on these mutants. We now report on the other female development defective mutants identified in our screening experiments. Co-segregation and complementation experiments were carried out on these mutants and we identified 80 genes that are required for female development (in addition to the 24 genes needed for anastomosis). We find that these genes fall into 5 general classes: 1) signal transduction pathway genes (25 genes), 2) transcription factor genes (7 genes), 3) chromatin remodeling genes (17 genes), 4) genes required for autophagy (11 genes), and 5) miscellaneous genes (20 genes). The PacC pathway genes are among the identified signal transduction pathway genes needed for female development. The activation of the PacC signal transduction pathway is a key signaling event in sexual development. Our experiments also suggest that autophagy and anastomosis are important for the movement of nutrients from the hyphal tissues supporting the developing perithecium.

129. *Aspergillus flavus* MAP kinase AflMpkB positively regulates developmental process but not aflatoxin production. Sang-Cheol Jun[1,2], Dong-Soon Oh[1], Jong-Hwa Kim[1], Kwang-Yeop Jahng[2], Kap-Hoon Han[1]. 1) Dept. of Pharmaceutical Engineering, Woosuk Univ, Wanju, Korea; 2) Div. of Biological Sciences, Chonbuk National University, Jeonju, Korea.

Developmental process of eukaryotes is controlled by the multiple regulatory systems including signal transduction pathways and transcription factors. One of the central signaling mechanisms includes mitogen-activated protein kinase (MAPK) pathway that transfer extracellular signals into nucleus, generating cellular responses. Previously, we have showed that *Aspergillus nidulans* MpkB, the yeast Fus3 MAP kinase ortholog, regulates sexual development and secondary metabolism. Here, we identified and characterized the ortholog of the *A. nidulans mpkB* gene in *Aspergillus flavus*, *AflmpkB*, to understand whether the *AflmpkB* gene has conserved function with *A. nidulans mpkB*. Deletion of *AflmpkB* did not affect hyphal growth but showed reduced conidia production Furthermore, *AflmpkB* null strain didn't produce any sclerotia while WT and recipient strain produced a lot of sclerotia in normal conditions. However, loss of *AflmpkB* resulted in normal aflatoxin biosynthesis, suggesting that the major function of *AflmpkB* is positive regulation of conidiation, sclerotia development but not mycotoxin production. These results indicate that *A. nidulans* and *A. flavus* MpkB have conserved and divergent roles in development and secondary metabolism.

130. Subcellular localization and kinase activity of GK4, a Phytophthora infestans GPCR-PIPK involved in actin cytoskeleton organisation. Chenlei Hua[1], Harold Meijer[1], Kiki Kots[1,2], Tijs Ketelaar[2], Francine Govers[1]. 1) Laboratories of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; 2) Laboratories of Cell Biology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

For dispersal and host infection plant pathogens largely depend on asexual spores. Pathogenesis and sporulation are complex processes that are governed by various cellular signaling networks including G-protein and phospholipid signaling. Oomycetes possess a family of novel proteins called GPCR-PIPKs (GKs) that are composed of a seven trans-membrane spanning (7-TM) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this domain structure GKs are anticipated to link G-protein and phospholipid signalling pathways. Our studies in the potato late blight pathogen Phytophthora infestans revealed involvement of one of twelve GKs (i.e. PiGK4) in spore development, hyphal elongation and infection. Moreover, ectopic expression in P. infestans of subdomains of PiGK1 and PiGK4 fused to a fluorescent protein showed that the GPCR domain targets the GKs to membranes surrounding different cellular compartments. To further elucidate the function of the PIPK domain we tested kinase activity of PiGK4 both in vivo and in vitro and analysed the relationship between PiGK4, phosphoinositide signaling and the organisation of the actin cytoskeleton using complementation in yeast combined with various live-cell markers.

131. External calcium ions and deletion of *per-1* gene suppressed the abnormal morphology of *och-1* and *frost* mutants in *Neurospora crassa*. Masayuki Kamei, Yuko Tsukagoshi, Shinpei Banno, Masakazu Takahashi, Akihiko Ichiishi, Makoto Fujimura. Faculty of Life Sciences, Toyo University, ORA-GUN, GUNMA, Japan.

Calcium ions play important roles in the growth and development in filamentous fungi. The *frost* mutant show slow growth and hyperbranching phenotypes that can be corrected by Ca^{2+} addition in *Neurospora crassa*. The *frost* gene is an ortholog of *S. cerevisiae cdc1* which encodes putative Mn^{2+}-dependent lipid phosphatase. We found that the abnormal morphology of the *och-1* mutant was quite similar to that of the *frost* mutant and its abnormality was also corrected by external Ca^{2+}. The *och-1* gene encodes an alpha-1,6-mannosyltransferase that is probably involved in sugar processing for GPI-anchor proteins. In yeast, the mutation of *per1* gene, encoding a protein is required for GPI remodeling pathway, suppresses the abnormal growth phenotype of *cdc1* mutant. To examine the effect of *per-1* gene, an ortholog of *per1* in *S. cerevisiae*, on the phenotypes of the *frost* and *och-1* mutants, we isolated two double mutants, *frost; Dper-1* and *och-1; Dper-1*. Although *per-1* gene disruptant showed the normal growth phenotype, *per-1* gene deletion significantly suppressed the slow growth and hyperbranching phenotypes of *frost* and also *och-1* mutants. Addition of Ca^{2+} did not affect the growth and morphology of the two double mutants. These results suggest the connection between FROST and OCH-1 may participate in lipid remodeling or calcium signaling in *Neurospora crassa*.

132. Functional analysis of carbonic anhydrases from the filamentous ascomycete *Sordaria macrospora*. Ronny Lehneck[1], Piotr Neumann[2], Achim Dickmanns[2], Ralf Ficner[2], Stefanie Pöggeler[1]. 1) Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August-University Göttingen; 2) Institute of Microbiology and Genetics, Department of Molecular Structural Biology, Georg-August-University Göttingen.

Carbonic anhydrases (CA) are widely distributed enzymes, which catalyzes the reversible hydration of carbon dioxide to bicarbonate and protons. Based on their amino acid sequence and structure, they can be divided into five distinct groups (a, b, g, d, x) which share no sequence similarity and have supposable evolved independently. All known fungal CAs belong either to the a-class or to the b-class. Our model organism *Sordaria macrospora* encodes at least four carbonic anhydrases: three of the b type, termed *cas1*, *cas2* and *cas3* (**c**arbonic **a**nhydrase of **S**ordaria) and one a-type, termed *cas4*. Previously, the functions of CAS1, CAS2 and CAS3 have been intensively studied (Elleuche and Pöggeler 2009) and displayed an involvement in fruiting-

body development and ascospore germination. Here, we present a functional characterization of the secreted a-CA CAS4. CAS4 seems to be involved in ammonium metabolism but not in ascospore germination. The Dcas4 mutant displayed a slightly reduced vegetative growth rate and a delayed fruiting-body development. Based on real time PCR analysis *cas4* is upregulated during the sexual development. Moreover, we present the phenotype of a quadruple mutant without any CAS genes. The complete CAS deletion strain (Dcas1/2/3/4) is able to grow under ambient air but the vegetative growth rate is drastically reduced and the mutant is only able to form thin hyphae. The mutant is even under elevated CO_2 levels (5 %) not able to form fruiting bodies. Heterologous expression in *Saccharomyces cerevisiae* demonstrated that CAS1 and CAS2 are active enzymes, but only CAS1 displays considerable in vitro activity. Furthermore, X-ray and gel filtration analyses revealed a tetrameric structure of CAS1 with a conserved histidine and two cysteine residues in the active center.

Elleuche and Pöggeler 2009: b-Carbonic anhydrases play a role in fruiting body development and ascospore germination in the filamentous fungus *Sordaria macrospora*; PLoS ONE. 2009; 4(4): e5177.

133. The *Coprinopsis cinerea cag1* (*cap-growthless1*) gene, whose mutation affects cap growth in fruiting body morphogenesis, encodes the budding yeast Tup1 homolog. H. Muraguchi, K. Kemuriyama, T. Nagoshi. Dept Biotechnology, Akita Prefectural Univ, Akita, Japan.

We have mutagenized a homokaryotic fruiting strain, #326, of *Coprinopsis cinerea* and isolated a mutant that fails to enlarge the cap tissue on the primordial shaft in fruiting. Genetic analysis of this mutant, *cap-growthless*, indicated that the mutant phenotype is brought about by a single gene, designated as *cag1*. The *cag1* locus was mapped on chromosome IX by linkage analysis using RAPD markers mapped to each chromosome. The *cag1* gene was identified by transformation experiments using BAC DNAs and their subclones derived from chromosome IX, and found to encode a homolog of *Saccharomyces cerevisiae* Tup1. The *Coprinopsis* genome includes another Tup1 homologous gene, designated *Cc.tupA*. Expression levels of these two *tup1* paralogs were examined using a real-time quantitative PCR method. *Cc.tupA* is predominantly expressed in vegetative mycelium. In contrast, in the cap tissue, transcript levels of *cag1* are similar to that of *Cc.tupA*. Since it is known that *S. cerevisiae* Tup1 forms homotetramer, interactions of Cag1 with itself and Cc.TupA were examined using yeast two-hybrid system. Cag1 interacts with itself through the N-terminal region and with Cc.TupA. Like Tup1, which interacts with Cyc8, the N-terminal region of Cag1 also interacts with the N-terminal region of Cc.Cyc8, which contains tetratricopeptide repeats. Based on expression and yeast two-hybrid analyses of Cag1 and Cc.TupA, combined with information on *S. cerevisiae* Tup1, we speculate that, in vegetative mycelium, Cc.TupA represses expression of genes required for cap growth, and Cag1, which might become expressed at the top of primordial shafts to produce the cap tissue and continue to be expressed in the cap tissue, might derepress and activate the expression through interaction with Cc.TupA.

134. Adaptation of the microtubule cytoskeleton to multinuclearity and chromosome number in hyphae of *Ashbya gossypii* as revealed by electron tomography. R. Gibeaux[1], C. Lang[2], A. Z. Politi[1], S. L. Jaspersen[3], P. Philippsen[2], C. Antony[1]. 1) European Molecular Biology Laboratory, Heidelberg, Germany; 2) Biozentrum, Molecular Microbiology, University of Basel, CH 4056 Basel, Switzerland; 3) Stowers Institute for Medical Research, Kansas City, USA.

The filamentous fungus Ashbya gossypii and the yeast Saccharomyces cerevisiae evolved from a common ancestor based on the high level of gene order conservation. Interestingly, A. gossypii lost the ability of cell divisions and exclusively grows as elongating multinucleated hyphae. Using electron tomography we reconstructed the cytoplasmic microtubule (cMT) cytoskeleton in three tip regions with a total of 13 nuclei and also the nuclear microtubules (nMTs) of four mitotic bipolar spindles. Each spindle pole body (SPB) nucleates three cMTs on average, similarly to S. cerevisiae SPBs. 80% of cMTs were growing as concluded from the structure of their plus-ends. Very long cMTs closely align for several microns along the cortex to generate dynein-dependent pulling forces on nuclei. The majority of nuclei carry duplicated side-by-side SPBs, which together emanate an average of six cMTs, in most cases in opposite orientation with respect to the hyphal growth axis. Such cMT arrays explain why many nuclei undergo short-range back and forth movements. Following mitosis, daughter nuclei carry a single SPB. The increased probability that all three cMTs orient in one direction explains the high rate of long-range nuclear bypassing observed in these nuclei. These results demonstrate how cMT arrays, despite a conserved number of microtubules, could successfully adapt to the demands of multinuclearity during evolution from mono-nucleated budding yeast-like cells to multinucleated hyphae. The modelling of A. gossypii mitotic spindles revealed a very similar structure to mitotic spindles of S. cerevisiae in terms of nMT number, length distribution and three-dimensional organisation even though A. gossypii carries 7 and S. cerevisiae 16 chromosomes per haploid genome. Our results suggest that the nMT cytoskeleton remained largely unaltered during the evolution and that two nMTs attach to each kinetochore in A. gossypii in contrast to only one in S. cerevisiae.

135. High resolution proteomics of spores, germlings and hyphae of the phytopathogenic fungus *Ashbya gossypii*. L. Molzahn[1,2], A. Schmidt[2], P. Philippsen[1]. 1) Biozentrum, Molecular Microbiology, University of Basel, CH4056 Basel, Switzerland; 2) Biozentrum, Proteomics Facility, University of Basel, CH4056 Basel, Switzerland.

Growth of the filamentous ascomycete A. gossypii is regulated by a genome very similar to the Saccharomyces cerevisiae genome even though the growth modes of both organisms differ significantly. During the previous decade progress was made to better understand some of these differences. 1. Cytokinesis in A. gossypii is not coordinated with mitosis and cell separation does not occur due to loss of specific genes which most likely led to the evolution of multinucleated hyphae. 2. Short nuclear cycle times and dynein-dependent pulling forces excerted on nuclei by autonomous cMT arrays with fast growing microtubules maintain a high nuclear density also in fast growing hyphae. 3. Polar growth sites once established support permanent and constantly accelerating polar surface expansion at hyphal tips at rates of up to 40mm2/min compared to 1mm2/min of yeast buds. Very efficient exocytosis and endocytosis could be documented in hyphal tips of A.gossypii. We want to understand on a system level the differences between both organisms and have started a proteomic approach. Total protein extracted from spores and developing A. gossypii hyphae was digested with trypsin, mixed with heavy isotope-labeled reference peptides and subjected to high resolution tandem MS analyses. We could identify 3900 proteins at each developmental stage. Significant quantitative changes of these proteins with respect to clusters of orthologous groups (COG) or gene ontology (GO) terms were identified during A.gossypii development and between log-phase growing S. cerevisiae cells and fast growing A. gossypii hyphae. Important differences concern ribosome biogenesis and translation, mitochondria biogenesis and respiration, glycolysis and gluconeogenesis, chromatin remodeling, chaperones, cell wall biosynthesis and the first reaction in several biosynthetic pathways.

136. Indoor Fungal Growth and Humidity Dynamics. Frank J.J. Segers[1], Karel A. van Laarhoven[2], Henk P. Huinink[2], Olaf Adan[2], Jan Dijksterhuis[1]. 1) Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; 2) Department of Applied Physics, Eindhoven University of Technology, Eindhoven, Netherlands.

Indoor fungi are present in a considerable part of the European dwellings and cause cosmetic and structural damage. The presence of indoor fungi poses a potential threat to human health as a result of continuous exposure as they are able to form allergens and mycotoxins. Indoor fungal growth does not exist without the presence and availability of water. Not much is known on the response of fungi to humidity dynamics during different stages of their development. Relative humidity (RH) and water activity (aw) are used in many studies for the amount of water available for the fungus. A RH of 80% or higher is thought to be required for fungal growth to occur. On average the RH is below 50% in normal buildings, suggesting a crucial role of humidity dynamics for fungal growth. In order to study the fungal response to humidity dynamics, two indoor fungal species, Cladosporium halotolerans and Penicillium rubens, were dried in controlled humidity vessels to stop growth and are rehydrated under high humidity conditions after a week. Non-linear Spectral Imaging Microscopy (NSIM) is a non-intrusive method to follow the response of fungal cells under varying relative humidity conditions by looking at the metabolic activity of separate cells. The different developmental stages of C. halotolerans and P. rubens before and after periods of a certain level of humidity are determined by using Cryo Scanning Electron Microscopy (CryoSEM). A different response to humidity dynamics was seen between several developmental stages and both fungi used. More in depth research will be done on the specific cellular response of the fungi to humidity dynamics.

137. Essentiality of Ku70/80 in Ustilago maydis is related to its ability to suppress DNA damage signalling at telomeres. Carmen de Sena-Tomas[1], Eun Young Yu[2], Arturo Calzada[3], William K. Holloman[2], Neal F. Lue[2], Jose Perez-Martin[1]. 1) IBFG (CSIC-USAL), Zacarias Gonzalez 3, 37007 Salamanca, Spain; 2) Cornell University Medical College, 1300 York Avenue, 10021 New York; 3) CNB (CSIC), Darwin 3, 28049 Madrid, Spain.

Ku heterodimer is formed of two subunits Ku70 and Ku80 that bind with high affinity to DNA ends in a sequence independent manner. Ku has a role in several cellular processes including DNA repair, telomere maintenance, transcription and apoptosis. Ku heterodimer is essential in human cells as well as in Ustilago maydis, a well-characterized fungal system used in DNA repair studies. We found that depletion of Ku proteins in U. maydis elicits a DNA damage response (DDR) at telomeres resulting in a permanent cell cycle arrest, which depends on the activation of the Atr1-Chk1 signalling cascade. A consequence of this inappropriate activation is the induction of aberrant homologous recombination at telomeres manifested by the formation of extrachromosomal telomere circles, telomere lengthening and the accumulation of unpaired telomere C-strand. Abrogation of the DDR response by deleting either chk1 or atr1 genes alleviates much of these aberrant recombination process suggesting that one of the roles of Ku proteins at telomeres in Ustilago maydis is related to the suppression of unscheduled DNA damage signalling at telomeres, in addition to the protection of telomeres.

138. *Magnaporthe oryzae* effectors with putative roles in cell-to-cell movement during biotrophic invasion of rice. Mihwa Yi[1], Xu Wang[2], Jung-Youn Lee[2], Barbara Valent[1]. 1) Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; 2) Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware 19711, USA.

Previous studies implicated rice plasmodesmata in two different aspects of rice blast disease caused by the hemibiotrophic ascomycetous fungus, *Magnaporthe oryzae*. First, effectors that are translocated into the cytoplasm of living rice cells move ahead into uninvaded host plant cells by a mechanism that depends on effector protein size and rice cell type. This suggested that these effectors move through plasmodesmata to prepare surrounding host cells for fungal infection. Second, biotrophic invasive hyphae (IH) search for locations to move into neighboring rice cells and they undergo extreme constriction when crossing the host cell wall. These findings and additional evidence suggested that IH manipulate host pit fields containing plasmodesmata for cell-to-cell movement. Our goals are to test these hypotheses, and to understand the molecular mechanisms responsible for cell-to-cell movement in blast disease. We have identified six biotrophy-associated secreted (Bas) proteins that accumulate around IH at the point where they have crossed the rice cell wall to invade neighboring rice cells. We designated these effectors as putative fungal movement proteins (fMPs). When imaged as fluorescently labeled fusion proteins, the fMPs show unique localization patterns at the cell wall crossing points. Functional analysis of the fMPs is underway. Precise microscopic characterization with correlative light and electron microscopy (CLEM) and time-course, live-cell imaging is being performed to decipher how the fungus manipulates the rice cell wall junction area for effector trafficking and its own cell-to-cell spread. The fMPs will be localized relative to each other and to plasmodesmata-specific fluorescent markers. We will compare the structure and function of rice plasmodesmata in invaded versus non-invaded rice cells. Our results will identify novel host targets exploited by the fungus and related infection mechanisms at the wall crossing sites to facilitate colonization in planta.

139. Functional characterization of autophagy genes *Smatg8* and *Smatg4* in the homothallic ascomycete *Sordaria macrospora*. Stefanie Poeggeler, Oliver Voigt. Genetics of Eukaryotic Microorganisms, Georg-August University, Göttingen, Germany.

Autophagy is a degradation process involved in various developmental aspects of eukaryotes. However, its involvement in developmental processes of multicellular filamentous ascomycetes is largely unknown. Here, we analyzed the impact of the autophagic proteins SmATG8 and SmATG4 on the sexual and vegetative development of the filamentous ascomycete *Sordaria macrospora*. A yeast complementation assay demonstrated that the *S. macrospora Smatg8* and *Smatg4* genes can functionally replace the yeast homologs. By generating homokaryotic deletion mutants, we showed that the *S. macrospora* SmATG8 and SmATG4 orthologs were associated with autophagy-dependent processes. *Smatg8* and *Smatg4* deletions abolished fruiting-body formation and impaired vegetative growth and ascospore germination, but not hyphal fusion. We demonstrated that SmATG4 was capable of processing the SmATG8 precursor. SmATG8 was localized to autophagosomes and SmATG4 was distributed throughout the cytoplasm of *S. macrospora*. Furthermore, we could show that *Smatg8* and *Smatg4* are not only required for nonselective macroautophagy, but for selective macropexophagy as well. Our results suggest that in *S. macrospora* autophagy seems to be an essential and constitutively active process to sustain high energy levels for filamentous growth and multicellular development even under nonstarvation conditions. (Voigt O, Pöggeler S Autophagy genes *Smatg8* and *Smatg4* are required for fruiting-body development, vegetative growth and ascospore germination in the filamentous ascomycete *Sordaria macrospora*. Autophagy. 2012 Oct 12;9(1). [Epub ahead of print]).

140. Laser microdissection and transcriptomics of infection cushions formed by *Fusarium graminearum*. Marike Boenisch[1], Stefan Scholten[2], Sebastian Piehler[3], Martin Münsterkötter[3], Ulrich Güldener[3], Wilhelm Schäfer[1]. 1) Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Germany; 2) Developmental Biology and Biotechnology, Biocenter Klein Flottbek, University of Hamburg, Germany; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Münich (GmbH), Neuherberg, Germany.

The fungal plant pathogen *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein) Petch) is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot of maize worldwide. Trichothecene toxins produced by the fungus e.g. nivalenol (NIV) and deoxynivalenol (DON) contaminate cereal products and are harmful to humans, animals, and plants. We demonstrated recently, that *F. graminearum* forms toxin producing infection structures during infection of wheat husks, so called infection cushions (Boenisch and Schäfer, 2011). The aims of the presented study were to further clarify the penetration mechanism of infection cushions by histological studies and to identify molecular characteristics of infection cushions by expression analysis. Structural characteristics of infection cushions were visualized by 3D images following laser scanning microscopy. We observed

multiple penetration events underneath infection cushions by scanning electron microscopy. Colonization of the underlying plant tissue was studied by bright field microscopy and transmission electron microscopy of LR-White serial sections. To understand the molecular basis of initial colonization of the leaf surface followed by infection cushion development, a laser capture microdissection (LCM) approach was established to isolate specifically runner hyphae and infection cushions. Several hundred runner hyphae and infection cushions grown on wheat glumes were collected using the PALM system (Zeiss) avoiding contamination with plant tissue. Total mRNA of runner hyphae and infection cushions were isolated and amplified. The cDNA library of each developmental stage was used for next generation sequencing with Illumina HiSeq 2000. Quantitative expression analysis show marked differences in gene expression patterns between runner hyphae and infection cushions. Different functional pathways specific for each infection stage were identified. Thereby new insights in the initial infection process of FHB disease are gained. To our knowledge, we provide the first transcriptome data of runner hyphae and infection cushions from a fungal plant pathogen obtained under *in planta* conditions. In summary, the power of combined microscopic and molecular approaches to analyze cell type-specific gene expression during fungal-plant-interactions is demonstrated.

141. Biochemical and biophysical analysis of the CarO rhodopsin of *Fusarium fujikuroi*. Jorge García-Martínez[1], Marta Castrillo[1], Javier Avalos[1], Ulrich Terpitz[2]. 1) Departamento de Genética, Universidad de Sevilla, Sevilla, Spain; 2) Lehrstuhl für Biotechnologie und Biophysik, Julius-Maximilians-Universität Würzburg, Biozentrum / Am Hubland, Würzburg, Germany.

Light controls many substantial processes in filamentous fungi, such as reproduction and pathogenicity. Fungi naturally possess light sensors, which react to a broad range of wavelengths with absorption maxima in the blue, green or red regions of the spectrum. Rhodopsins are green light-absorbing membrane-integrated photoreceptors consisting of seven transmembrane helices forming an interior pocket for the chromophore, either all-trans or 11-cis retinal, covalently bound to the protein via a protonated Schiff-base. Type I rhodopsins, predicted to bind all-trans retinal, are widespread in ascomycota and basidiomycota. Upon light-activation, type I rhodopsins act as proton pumps or sensory proteins; however, detailed knowledge of their physiological function and biological role in fungi is still missing. The gibberellin-producing fungus *Fusarium fujikuroi* contains two rhodopsin encoding genes, *carO* and *opsA*, whose mutations produce no external phenotypic alterations. The *carO* gene is linked and co-regulated with genes coding for enzymes for retinal-synthesis, whose expression is strongly induced by light. To gain information on CarO biological role, we have combined biophysical methods to analyse the localisation and function of this rhodopsin in *F. fujikuroi* mycelia. We established a strain expressing CarO fused to a yellow fluorescent protein (YFP) under control of the *carO* promoter. This strain was investigated with confocal laser scanning microscopy (cLSM) and super-resolution fluorescence imaging (dSTORM) to reveal the subcellular localisation of CarO. Protein-localisation was compared with data recorded from a *S. cerevisiae* DSY5 strain overexpressing CarO-YFP. Additionally, the carO-YFP gene fusion was expressed in neuroblastoma cells, where it exhibited an efficient ion pump-activity, as demonstrated by Patch-clamp techniques. The results suggest a light dependent ion-pumping role in the fungus, nonessential under standard laboratory conditions.

142. Roles of membrane and organellar calcium channels and transporters in controlling pulsatile $[Ca^{2+}]_c$ signatures. Hye-Seon Kim[1], Jung-Eun Kim[2], Kirk Czymmek[1], Robert Cirino[1], Randall Duncan[1], Hokyoung Son[3], Yin-Won Lee[3], Seogchan Kang[2]. 1) Department of Biological Sciences, University of Delaware, Newark, DE 19711; 2) Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802; 3) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921.

Calcium ions translate diverse environmental stimuli into many different physiological and developmental functions in fungi via an evolutionary conserved cell-signaling pathway. Using the expression of Yellow Cameleon YC3.60, a fluorescent protein-based, ratiometric Ca^{2+} sensor in *Magnaporthe oryzae*, *Fusarium oxsyporum*, and *F. graminearum*, we reported that cytoplasmic tip high Ca^{2+} signatures exhibited distinct species-specific and age-dependent pulsatile patterns (FGB 49:589). We successfully expressed a new circularly permuted Ca^{2+} sensor, GCaMP5, in *F. graminearum* and *F. oxysporum* and GCaMP3 in *Neurospora crassa*. The improved sensitivity, photostability, and fast kinetics of GCaMP5 enabled us to image smaller Ca^{2+} changes in hyphae tips with high-speed imaging that showed that the tip high Ca^{2+} gradient has multiple origins. Disruption of *F. graminearum* genes encoding plasma membrane Ca^{2+} channels (*Mid1*, *Cch1*, and *Fig1*), vacuole/ER Ca^{2+} pumps (*Pmc*, *Pmr*), calcineurin transcription factor (*Crz1*), and vacuole H^+/Ca^{2+} exchanger (*Vcx1* and *Vcx2*) significantly altered the amplitude, interval, and origin of Ca^{2+} pulses and also affected growth. Additional phenotypes associated with these mutants are currently being characterized. The combination of molecular genetics, genomics, live cell imaging, and correlative microscopy will help us study the mechanism underpinning fungal Ca^{2+} signaling at multiple scales ranging from the function and mode-of-action of individual genes to nano-scale dynamics of individual proteins and subcellular machineries.

143. Characterization of positive regulator for asexual and sexual reproduction in the cereal head blight pathogen *Gibberella zeae*. Jungkwan Lee[1], Boknam Jung[1], Hokyoung Son[2], Yin-Won Lee[2]. 1) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; 2) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea.

Gibberella zeae is an important plant pathogen that causes cereal head blight and produces mycotoxins that are harmful to animals and humans. Ascospores and conidia contribute to the primary inoculums and propagation for disease epidemics. In this study, we identified one putative C2H2 zinc finger transcription factor (*prd1*) that is required for both conidiation and sexual reproduction, as screening transcription factor mutant collection we previously generated. *prd1* deletion mutants impaired conidial production and lost both self-fertility and female fertility, but retain male fertility. The overexpression of the gene increased the amount of conidial production and resulted in earlier maturation of fruiting body formation than the wild-type strain. The vegetative growth of deletion and overexpression mutants was increased and decreased on nutrient-rich mediua, respectively, but was not different from the wild-type strain on nutrient-poor media. This study was the first report for transcription factor which positively regulates both conidiation and sexual reproduction, and the characterization of genes regulated by this gene will be further studied.

144. Functional analysis of Elongator complex protein 3 in *Gibberella zeae*. Y. J. Lee[1], H. Son[1], J.-C. Kim[2], G. J. Choi[2], Y.-W. Lee[1]. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Eco-friendly New Materials Research Group, Research Center for Biobased Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea.

Gibberella zeae (anamorph: *Fusarium graminearum*) is a causal agent of Fusarium head blight (FHB) which causes huge economic losses in cereal crops such as wheat and barley. In addition to yield reduction, mycotoxin contamination of grain presents a threat to human safety. We examined one of Restriction-Enzyme-Mediated Integration (REMI) mutants Z43R9282 showing defects in virulence and sexual development and identified a gene encoding Elongator complex protein 3 (ELP3). ELP3 is a catalytic subunit of Elongator complex and contains histone acetyltransferase (HAT) domain. The biological function of *ELP3* gene was studied by targeted deletion in *G. zeae*. Deletion of *ELP3* resulted in retarded growth and delay of sexual development compared to the wild-type strain. Most of the ascospores had two cells in the *ELP3* deletion mutants, while wild-type ascospores usually had four cells. The

length of the mutant conidia was approximately 25% longer than the wild type. Deletion mutants of *ELP3* were sensitive to stress conditions, such as high-salt stress (NaCl and KCl), suggesting a role in adaptation to environmental condition. Virulence on wheat heads was greatly reduced in the *ELP3* deletion mutants. These results demonstrate that *ELP3* is required for normal sexual and asexual development and *ELP3* could be involved in cell size regulation in *G. zeae*.

145. Functional analyses of regulators of G protein signaling (FgRGS) and GzGPA proteins in *Gibberella zeae*. A.R. Park[1], A.-R. Cho[1], J.-A. Seo[2], K. Min[1], H. Son[1], J. Lee[3], G.J. Choi[4], J.-C. Kim[4], Y.-W. Lee[1]. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Science and Technology Division, Ministry for Food, Agriculture, Forestry and Fisheries, Gyeonggi-Do 427-712, Republic of Korea; 3) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; 4) Eco-friendly New Materials Research Group, Research Center for Biobased Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea.

G protein signaling pathways play key roles in the regulation of fungal development, secondary metabolism, and virulence. Regulators of G protein signaling (RGS) proteins make up a highly diverse and multifunctional protein family that plays a critical role in controlling heterotrimeric G protein signaling. The genome of the plant pathogenic fungus *Gibberella zeae* contains seven *RGS* genes (*FgFlbA, FgFlbB, FgRgsA, FgRgsB, FgRgsB2, FgRgsC, and FgGprK*). Here we functionally characterized the function of these genes in various cellular processes. Mutant phenotypes were observed for deletion mutants of *FgRgsA* and *FgRgsB* in vegetative growth, *FgFlbB* and *FgRgsB* in conidia morphology, *FgFlbA* in conidia production, *FgFlbA*, *FgRgsB*, and *FgRgsC* in sexual development, *FgFlbA* and *FgRgsA* in spore germination and mycotoxin production, and *FgFlbA*, *FgRgsA*, and *FgRgsB* in virulence. Furthermore, *FgFlbA*, *FgRgsA*, and *FgRgsB* acted pleiotropically, while *FgFlbB* and *FgRgsC* deletion mutants exhibited a specific defect in conidia morphology and sexual development, respectively. Site-directed Ga subunits mutagenesis and overexpression of the *FgFlbA* gene revealed that deletion of *FgFlbA* and dominant active GzGPA2 mutant, *gzgpa2*^{Q207L}, had similar phenotypes in cell wall integrity, perithecia formation, mycotoxin production, and virulence, suggesting that FgFlbA may regulate asexual/sexual development, mycotoxin biosynthesis, and virulence through GzGPA2-dependent signaling in *G. zeae*. Especially, GzGPA2 might activate trichothecene production in a FgFlbA-dependent manner.

146. A novel gene, *GEA1*, is required for ascus cell wall development in the ascomycete fungus, *Gibberella zeae*. H. SON[1], J. Lee[2], Y.-W. Lee[1]. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea.

The ascomycete fungus *Gibberella zeae* is a devastating plant pathogen for major cereal crops. Ascospores are produced via sexual reproduction and forcibly discharged from mature perithecia, which function as the primary inocula. Perithecium development involves complex cellular processes and is under polygenic control. In this study, a novel gene, *GEA1*, was found to be required for ascus wall development in *G. zeae*. *GEA1* deletion mutants produced normal-shaped perithecia and ascospores, yet ascospores were observed to precociously germinate inside of perithecium. Moreover, *GEA1* deletions resulted in abnormal ascus walls that collapsed prior to ascospore discharge. Based on localization of GEA1 to the endoplasmic reticulum (ER), GEA1 may be involved in protein export from the ER to the ascus wall biogenesis. This is the first report to identify a unique gene required for ascus wall development in *G. zeae*.

147. A systems-biology approach to build gene-regulatory network models connecting osmotic stress responses and asexual development in *Fusarium graminearum*. A. Thompkins, M. Sexton, S. Atkinson, B. Bass, E. Delancy, J. Rhodes, J. Flaherty. Science and Mathematics, Coker College, Hartsville, SC.

Fusarium graminearum is a notorious fungal plant pathogen and causes head blight disease in small grain cereals and ear rot disease in maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Mycotoxin formation and asexual development are thought to share common nodes of genetic regulation. However, the regulatory networks connecting salt/osmotic stress to either is limited or undefined. Salt tolerance is a complex trait that remains poorly understood. Very few genes have been identified that are required for salt tolerance in plants, animals, or fungi. To address this, we screened >5,000 insertional mutants of *F. graminearum* (PH-1) for gain-of-function or loss-of-function phenotypic classes specific to both asexual development (conidiation) and osmotic stress responses. These screens yielded strains representing all classes and one outlier from each were chosen for additional analyses. Mutant 9E1 exhibits an “osmotic hyper-tolerant” phenotype when cultured on growth media containing either NaCl or glycerol. In contrast, mutant 11B1 displays an “osmotic-overly sensitive” phenotype, where growth is severely limited on concentrations of solute that have a negligible effect on growth by control strains. Both 9E1 and 11B1 grow normally on non-osmotically adjusted media and were subsequently chosen for transcription-profiling experiments. Additionally, mining gene expression data of developmental mutants 8B5 (aconidial) and 8E8 (hyperconidial) have revealed coordinately expressed, putative candidate regulatory genes. Based on a transcriptomics framework, we applied a bioinformatics approach to identify shared gene regulatory networks involved in osmotic stress responses and conidiation.

This project was supported in part by grants from the National Science Foundation (MCB 0845324), the National Center for Research Resources (5 P20 RR016461), and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

148. Starvation enhances heterokaryon formation between incompatible strains of *Fusarium oxysporum*. Shermineh Shahi, Martijn Rep. Molecular Plant Pathology, Swammerdam Institute for Life Sciences, Amsterdam, Nordholland, Netherlands.

Fusarium oxysporum (Fo) is a pathogenic species complex with a broad host range. Comparative genomics revealed lineage-specific (LS) genomic regions in *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) that account for more than 25% of the genome. At least two LS chromosomes can be transferred horizontally to non-pathogenic *Fo* strains, resulting in acquired pathogenicity in the recipient [1]. Here we want to elucidate the chromosome transfer pathway and the mechanisms by which the incompatibility reaction between strains is avoided. It has been suggested that heterokaryon formation is necessary for horizontal chromosome transfer in *Colletotrichum gloeosporioides* [2] and that heterokaryon incompatibility is suppressed after conidial anastomosis tube (CAT) fusion [3]. To study nuclear dynamics during formation of heterokaryotic cells in *Fo*, we observed green or red fluorescent protein labeled nuclei of *Fol* and a non-pathogenic *Fo* strains in a vegetatively incompatible interaction.

While in rich medium co-cultivation of both strains revealed no heterokaryotic cells, co-cultivation under starvation conditions led to up to ~30% heterokaryotic colonies (red and green nuclei). We were able to distinguish between different types of heterokaryotic conidia. In some cases after germination only one of the nuclei was able to propagate, which always originated from the pathogenic strain. In other cases both nuclei were able to propagate and these colonies in turn produced uninucleate conidia (yellow nuclei). Another intriguing finding was that the pathogenic strain used fared better under starvation conditions (higher germination and growth rate). We conclude that under starvation condition *Fol* is the dominant/fitter strain and that heterokaryon formation in *Fo* is greatly enhanced, possibly by further suppressing non-self recognition machinery in CATs and/or increased hyphal

fusion. We hypothesize that starvation might be a driving force for horizontal chromosome transfer in order to increase the chance of survival.

Reference:[1] Ma, L.-J. et al; Nature 464, 367-373 (2010) [2] Manners, JM & He,C; Mycol Progress 10:383-388 (2011) [3] Ishikawa, FH et al; PLoS ONE 7(2): e31175. doi:10.1371/journal.pone.0031175 (2012).

149. Requirements for horizontal chromosome transfer in the plant pathogenic fungus Fusarium oxysporum. Ido Vlaardingerbroek, Martijn Rep. FNWI, University of Amsterdam, Amsterdam, Netherlands.

Strains within the *Fusarium oxysporum* species complex are clonal and diverse. A number of them are pathogenic to plants but rarely can they infect more than one host. Host specificity is determined by the presence of a set of secreted effector genes. These genes typically reside on Lineage Specific (LS) chromosomes that can be transferred between strains, even if they are vegetatively incompatible. These extra chromosomes typically carry no housekeeping genes and have many more transposable elements then the non-LS or core chromosomes. If a strain receives one of these chromosomes it can acquire the ability to infect a new host, compatible with the effector genes the chromosome harbours. Our main interests at this moment are (1) determining which chromosomes are amenable for transfer, and (2) which cellular processes are involved in transfer. To determine which chromosomes can be transferred, we created a bank of random insertional mutants carrying an antibiotic resistance marker. These have been tested for chromosome transfer. A few of these showed consistent transfer of the chromosome tagged with the marker. By screening a large number of transformants we should cover the entire genome. In addition to the screen we tagged an LS chromosome that we know can be transferred, as well as the smallest of the core chromosomes, with GFP. In this way we can directly compare transfer capability of these chromosomes. We hypothesize that the LS chromosomes' unique make-up is required for transfer. We will also test these strains for stability of the tagged chromosome by screening spores for the loss of GFP expression using FACS. In this way we can test whether transferrable (LS) chromosomes differ in stability from core chromosomes under varying conditions. To identify cellular processes involved in chromosome transfer, we are making deletion mutants for genes required for cellular processes we suspect might be involved in chromosome transfer. These will be tested for transfer efficiency compared to the wild-type strains. We are currently investigating hyphal fusion, heterochromatin formation and programmed cell death. By combining the results from these two research lines we should be able to discover which chromosomes can be transferred as well as the chromosomal features and processes involved.

150. Characterization of the endocytotic proteins Yel1-Arf3-Gts1 in *Ashbya gossypii* and the role of Gts1 in endocytosis, actin localization and filamentous growth. Therése Oskarsson, Klaus Lengeler, Jürgen Wendland. Carlsberg Laboratory, Copenhagen, Denmark.

Endocytic vesicle formation and regulation thereof is performed by a complex protein machinery, coordinating every detail of the endocytic process from initiation and pit formation to vesicle scission and uncoating.

We have used the filamentous fungi *Ashbya gossypii* to study three proteins that are involved in uncoating of vesicles in clathrin-mediated endocytosis. We deleted the corresponding genes encoding the GTP-binding protein Arf3 and its regulators; the Guanine nucleotide Exchange Factor Yel1 and the ArfGAP protein Gts1, using PCR-based gene targeting methods. We then characterized these mutant strains under various conditions.

While no deletion-specific phenotypes could be observed in the *Darf3* and *Dyel1*, the *Dgts1* strain shows several severe mutant phenotypes. Deletion of *GTS1* results in a strong growth defect and renders mycelia with severe endocytotic deficiencies indicated by distinctly reduced endocytic rates, and large immobile vacuoles. Other phenotypic observations in *A. gossypii Dgts1* strains indicate that Gts1 may have additional functions other than regulating the activity of Arf3. We have observed effects of Gts1 on temperature stress resistance, actin localization and polar- as well as filamentous growth.

The importance of *GTS1* for polarized hyphal growth leads us to studying the *GTS1* homolog of the human fungal pathogen *Candia albicans* in an effort to elucidate its role for the yeast-to-hyphal transition in this dimorphic fungi.

151. A Late Embryogenesis Abundant (LEA) protein in *Neosartorya fischeri* confers protection against desiccation. Martin Richard van Leeuwen, Timon T Wyatt, Tineke M van Doorn, Jan Dijksterhuis. Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

Late Embryogenesis Abundant (LEA) proteins were first characterized in cotton and wheat and are synthesized in abundance during the late maturation stage of seed development. As the seed matures, water content decreases greatly inducing severe desiccation stress. Expression of LEA proteins is linked to the acquisition of desiccation tolerance. Using BLAST to search for LEA like proteins in various filamentous fungal genomes (*Aspergillus niger*, *Aspergillus flavus*, *Emericella nidulans*, *Penicillium chrysogenum*, *Talaromyces stipitatus* and *Neosartorya fischeri*) resulted in orthologs in each mentioned species, indicating the wide spread appearance of LEA proteins in fungi. Ascospores produced by *N. fischeri* are able to survive long periods under various stressors. However, deletion of the LEA gene resulted in diminished tolerance against desiccation and high temperatures. In addition, heterologous expression of LEA in *Escherichia coli* conferred increased tolerance against osmotic- and salt stress. Interestingly, LEA was able to function as protectant for enzymes that normally lose activity under influence of stress. Lactate dehydrogenase (LDH) was inactivated by heat stress and freeze-thaw cycles. In the presence of LEA, LDH activity was maintained. Our results show that LEA are wide spread in filamentous fungi and function in tolerance against stressors like heat, freeze-thaw and desiccation. LEA could play an important role in stress tolerance of survival propagules like ascospores and conidia.

152. Coordination of polarized secretion by the exocyst complex is critical for filamentous growth and cytokinesis in *Ustilago maydis*. Michaela Wehr[1], Kay Oliver Schink[2], Michael Bölker[1]. 1) Philipps University, FB Biologie, AG Boelker Marburg, Hessen, Germany; 2) Department of Biochemistry, Institute for Cancer Research The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway.

To establish and sustain their polarity, cells have to transport proteins and membrane lipids to defined locations at the growing tip. This is achieved by directional transport of vesicles that fuse with the plasma membrane. Vesicle fusion and active exocytosis requires the presence of an octameric protein complex, the exocyst. In *S. cerevisiae*, two proteins of the exocyst complex, Sec3 and Exo70, were shown to serve as landmark proteins for exocytosis. The other components of the exocyst tether secretory vesicles carrying the Rab GTPase Sec4 to the membrane. Fusion of secretory vesicles occurs via interaction of the exocyst with SNARE proteins. To elucidate the function and regulation of the exocyst complex and its associated proteins in *Ustilago maydis*, we have characterized the Rab GTPase Sec4 and the exocyst proteins Sec3, Exo70 and Sec15 by genetic, cell biological and biochemical approaches. We found that of the two landmark proteins, only one is important for polar growth in *U. maydis*. Interestingly, this gene is not essential, suggesting that in *U. maydis* exocytosis sites can be also marked by alternative mechanisms. Another essential player for polar growth in *U. maydis* is the exocyst subunit Sec15, which mediates the interaction of the exocyst with incoming secretory vesicles. Conditional mutants of *sec15* are defective in hyphal tip growth and are affected in long-distance transport of secretory vesicles. In contrast to *S. cerevisiae* where Sec4 vesicles are transported along the actin cytoskeleton, long distance transport of vesicles depends in *U. maydis* on the microtubule cytoskeleton. Furthermore, we studied mutants of different motor proteins to get insights into the molecular mechanisms of secretory vesicle trafficking.

153. Localization of Neurospora crassa Cell Fusion Proteins. Ci Fu, Stephen J. Free. Biological Sciences, University at Buffalo, Buffalo, NY.

A screen of mutants in Neurospora crassa single gene deletion library identified 24 cell fusion genes. Bioinformatics studies indicate that 14 of these genes are likely to function in signal transduction pathways, 4 genes are transcription factors, 3 genes are likely to be involved in the process of vesicular trafficking, and 3 genes are highly conserved in fungal species with unknown functions. GFP and RFP fusion proteins were constructed for 2 vesicular trafficking proteins AMPH-1 and HAM-10, and 1 conserved hypothetical protein HAM-8 to study their functions during cell fusion process. Fluorescent protein markers for cellular organelles (including nucleus, mitochondria, golgi apparatus, endoplasmic reticulum, vacuole and vesicle), and for cytoskeleton (including actin filament and microtubule) were obtained from Fungal Genetic Stock Center. Strains expressing individual fluorescent protein marker were used to study the cellular localizations of AMPH-1, HAM-10 and HAM-8 by using fluorescent confocal microscopy. The fluorescent protein marker strains were also used to study the dynamics of organelle movements during cell fusion by using time-lapse fluorescence microscopy. Fluorescent signals from AMPH-1, HAM-10 and HAM-8 were compared with two signaling molecules MAK-2 and SO to study their potential involvement in signal transduction. Results shown AMPH-1, HAM-8 and HAM-10 all colocalize with vesicle marker. One of the conserved hypothetical proteins, HAM-6, was modified with a FLAG tag to study its functions during cell fusion.

154. Identification of novel *Neurospora crassa* genes involved in hyphal fusion by tanscriptomic analysis. Wilfried Jonkers, Abigail C. Leeder, N. Louise Glass. Department of Plant and Microbial Biology, University of California, Berkeley, CA.

Hyphal fusion of *Neurospora crassa* germlings is a highly regulated process involving -among others- the conserved MAP kinase MAK-2 and the SO protein of unknown biochemical function. During chemotrophic interactions between two genetically identical germlings, MAK-2 and SO alternately localize at the conidial anastomosis tubes (CATs) every 4 minutes, perfectly out of phase of each other. How this process is initiated, maintained and what other proteins are involved is still unknown. One conserved fungal target of MAK-2 is the yeast Ste12-like transcription factor, named PP-1. Similar to *mak-2*, *pp-1* is also required for hyphal fusion and normal mycelial growth. To identify downstream targets of MAK-2 and PP-1 that may play a role in germling fusion, micro-array and RNAseq analyses were performed on wild type (WT) and D*pp-1* strains. Combining the micro-array and RNAseq data, 32 genes were identified that showed at least 2-fold differential expression in WT as compared to D*pp-1*. These include six genes, which are homologs of yeast Ste12 targets. To test the involvement of these genes in hyphal fusion, a deletion strain was obtained or constructed and assayed for germling fusion phenotype. Three deletion strains were completely devoid of fusion: D*ham-7*, D*asm-1* and D*ham-11*, and one deletion strain, D*ham-12* showed reduced fusion frequencies when compared to WT. *ham-7* was previously identified as fusion gene while *asm-1* was shown to be involved in meiosis. When D*ham-7* + D*ham-7* or D*ham-7* + WT germlings are confronted which each other, chemotropic interactions are not initiated, CATs are not observed and MAK-2 and SO are localized predominantly to the cytoplasm. *ham-11* is a newly identified gene involved in germling fusion; D*ham-11* + D*ham-11* germlings do not show chemotropic interactions or cell fusion. However, in contrast to D*ham-7*, D*ham-11* germling fuse normally with WT germlings. MAK-2 and SO also show normal oscillation in WT and D*ham-11* germlings undergoing chemotropic growth. The observations suggest that HAM-11 might be involved in the production or proper release of a signal capable of inducing cell recognition and the germling fusion process.

155. *N*-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi. Sarah A. Gilmore[1], Shamoon Naseem[2], James B. Konopka[2], Anita Sil[1]. 1) Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY.

Cellular differentiation is an essential process for the development and growth of multicellular eukaryotic organisms. Similarly, many unicellular organisms undergo a program of cellular differentiation to produce a new cell type specialized for survival in a distinct environmental niche. Systemic dimorphic fungal pathogens, such as *Histoplasma capsulatum* (*Hc*) and *Blastomyces dermatitidis* (*Bd*), can switch between a unicellular parasitic yeast form adapted for growth within mammals and an infectious soil-growing filamentous form as part of their natural life cycles. Temperature is thought to be the predominant environmental cue that promotes cellular differentiation of systemic dimorphic fungi; however, work with other fungi indicates that additional environmental cues including CO2, light, and nutrient availability can influence how an organism responds to its environment. Recent work suggests that the ubiquitous monosaccharide *N*-acetylglucosamine (GlcNAc) can play a role in cell signaling in fungi. We identified GlcNAc as a potent inducer of the yeast-to-filament transition in *Hc* and *Bd*. Micromolar concentrations of exogenous GlcNAc were sufficient to induce a robust morphological transition of *Hc* yeast cells to filamentous cells at room temperature, indicating that dimorphic fungal cells may be sensing GlcNAc, or one of its catabolic byproducts, to promote filamentation. Using GlcNAc as a tool to induce a robust and more synchronous phase transition of *Hc* yeast cells to filaments, we examined the temporal regulation of the *Hc* transcriptome during morphogenesis to reveal candidate genes involved in establishing the filamentous growth program. Two genes we identified during transcriptome analysis included *NGT1* and *NGT2*, which encode GlcNAc major facilitator superfamily transporters. RNAi depletion of *NGT1* or *NGT2* rendered *Hc* cells unable to respond to exogenous GlcNAc. Furthermore, wild type levels of *NGT1* and *NGT2* transcripts were important for efficient *Hc* yeast-to-filament conversion even in the absence of exogenously added GlcNAc. These data suggest that Ngt1 and Ngt2 may monitor endogenous GlcNAc as part of an autoregulatory system that allows *Hc* to regulate its filamentous growth.

156. How water influences fungal growth on "real" materials. H.P. Huinink[1], K.A. Laarhoven, van[1], M. Bekker[1], J. Dijksterhuis[2], O.C.G. Adan[1]. 1) Applied Physics, Eindhoven University of Technology, Eindhoven, Netherlands; 2) CBS - KNAW, Utrecht, Netherlands.

Understanding fungal growth on construction materials is important to control problems with mould growth in buildings. The indoor environment is generally a harsh environment for a fungus. The climate is relatively dry and only during certain events at specific locations in the building (cooking, showering, etc.) there are peaks in the humidity. The porous nature of construction materials seems to play an important role in the survival of organisms, because it buffers the climate at the surface of materials by storing water. A model has been developed that describes the thermodynamic state and flow of water inside porous materials in connection to the growth of the organism. The model shows that the activity of water in a material is the key parameter controlling growth. However, the model also proves that growth cannot be predicted on the basis of experiments performed on idealized microbiological media (agar) with a well defined water activity. In those media water is always abundantly present irrespective of the activity. In porous materials however the amount of water dramatically reduces with the water activity. It is shown that porous materials with small pores in general contain more water than materials with big pores. A drop in the amount of water due to a decreasing activity has direct consequences for the food supply. Whereas in idealized media the amount of water is very high and therefore the mobility of nutrients, in porous materials the mobility of nutrients will decrease with decreasing water activity. To understand the behavior of a fungus on materials, its growth has to be really studied on these materials.

157. Identification and characterization of two genes required in the control of a cell degeneration in the filamentous fungi *Podospora anserina*. Herve Lalucque[1,2], Fabienne Malagnac[1,2], Pierre Grognet[1,2], Philippe Silar[1,2]. 1) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de demain (LIED), 75205 Paris France; 2) Institut de Génétique et Microbiologie (IGM), UMR 8621 CNRS Univ Paris Sud, 91405 Orsay France.

For several years, we use the coprophilous fungus *Podospora anserina* to study a cell degeneration called Crippled Growth (CG) triggered by an

epigenetic and cytoplasmic element. In the wild-type strain, this element is produced during stationary phase and eliminated at growth renewal. However, in some particular growth conditions, the element is not eliminated in growing hyphae triggering CG. Previous results showed that CG is controlled by two MAPK modules, the PaNox1 NADPH oxidase and IDC1, a protein with unknown activity. Here, we describe the identification and characterization of two new partners involved in the control of CG, IDC2 and IDC3. Data show that IDC2 and IDC3 likely act downstream of PaNox1 to regulate the paMpk1 MAPK. We will present a thorough analysis of the phenotypic of the *IDC2* and *IDC3* mutants and the phylogenetic studies of the IDC2 and IDC3 proteins.

158. Dynein drives oscillatory nuclear movements in the phytopathogenic fungus *Ashbya gossypii* and prevents nuclear clustering. S. Grava, M. Keller, S. Voegeli, S. Seger, C. Lang, P. Philippsen. Biozentrum, Molecular Microbiology, University of Basel, CH 4056 Basel, Switzerland.

In the yeast Saccharomyces cerevisiae the dynein pathway has a specific cellular function. It acts together with the Kar9 pathway to position the nucleus at the bud neck and to direct the pulling of one daughter nucleus into the bud. Nuclei in the closely related multinucleated filamentous fungus Ashbya gossypii are in continuous motion and nuclear positioning or spindle orientation is not an issue. A. gossypii expresses homologues of all components of the Kar9/Dyn1 pathway, which apparently have adapted novel functions. Previous studies with A. gossypii revealed autonomous nuclear divisions and, emanating from each MTOC, an autonomous cytoplasmic microtubule (cMT) cytoskeleton responsible for pulling of nuclei in both directions of the hyphal growth axis. We now show that dynein is the sole motor for bidirectional movements. Surprisingly, deletion of Kar9 shows no phenotype. Dyn1, the dynactin component Jnm1, the accessory proteins Dyn2 and Ndl1, and the potential dynein cortical anchor Num1 are involved in the dynamic distribution of nuclei. In their absence, nuclei aggregate to different degrees, whereby the mutants with dense nuclear clusters grow extremely long cMTs. Like in budding yeast, we found that dynein is delivered to cMT +ends, and its activity or processivity is probably controlled by dynactin and Num1. Together with its role in powering nuclear movements, we propose that dynein also plays (directly or indirectly) a role in the control of cMT length. Those combined dynein actions prevent nuclear clustering in A. gossypii and thus reveal a novel cellular role for dynein.

159. Quantification of the thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography. Karen Stephenson[1], Fordyce Davidson[2], Neil Gow[3], Geoffrey Gadd[1]. 1) Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, United Kingdom; 2) Division of Mathematics, University of Dundee, Dundee, United Kingdom; 3) Institute of Medical Sciences, University of Aberdeen, Aberdee, United Kingdom.

Thigmotropism is the ability of an organism to exhibit an orientation response to a mechanical stimulus. We have quantified the thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography. We show that mutants that lack the formin BNI-1 and the Rho-GTPase CDC-42, an activator of BNI-1, had an attenuated thigmotropic response. In contrast, null mutants that lacked cell end-marker protein TEA-1 and KIP-A, the kinesin responsible for its localisation, exhibited significantly increased thigmotropism. These results indicate that vesicle delivery to the hyphal tip via the actin cytoskeleton is critical for thigmotropism. Disruption of actin in the region of the hyphal tip which contacts obstacles such as ridges on microfabricated slides may lead to a bias in vesicle delivery to one area of the tip and therefore a change in hyphal growth orientation. This mechanism may differ to that reported in *Candida albicans* in so far as it does not seem to be dependent on the mechanosensitive calcium channel protein Mid1. The *N. crassa* D*mid-1* mutant was not affected in its thigmotropic response. Although it was found that depletion of exogenous calcium did not affect the thigmotropic response, deletion of the *spray* gene, which encodes an intracellular calcium channel with a role in maintenance of the tip-high calcium gradient, resulted in a decrease in the thigmotropic response of *N. crassa*. This predicts a role for calcium in the thigmotropic response. Our findings suggest that thigmotropism in *C. albicans* and *N. crassa* are similar in being dependent on the regulation of the vectorial supply of secretory vesicles, but different in the extent to which this process is dependent on local calcium-ion gradients.

160. Specificity determinants of GTPase recognition by RhoGEFs in *Ustilago maydis*. Britta A.M. Tillmann[1], Kay Oliver Schink[2], Michael Bölker[1]. 1) Philipps-Universität Marburg FB Biologie, AG Bölker Karl-von-Frisch-Str. 8 35032 Marburg, Germany; 2) Department of Biochemistry, Institute for Cancer Research The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway.

Small GTPases of the Rho family act as molecular switches and are involved in the regulation of many important cellular processes. They are activated by specific guanine nucleotide exchange factors (Rho-GEFs). Rho-GTPases interact in their active, GTP-bound state with downstream effectors and trigger various cellular events. The number of Rho-GEFs and downstream effectors exceeds the number of GTPases. This raises the question how signalling specificity is achieved. In recent years it became evident that correct signalling depends on both the specificity of the activating Rho-GEF and on scaffolding proteins that connect the activators with specific downstream effectors. Here, we analysed the Cdc42-specific *U.maydis* Rho-GEFs Don1, Its1 and Hot1and the Rac1-specific Rho-GEF Cdc24 for their role in Cdc42 and Rac1 signalling both *in vivo* and *in vitro*. We observed that the recognition mechanisms for Cdc42 differ between Hot1 and the other Cdc42-specific Rho-GEFs. While a single amino acid at position 56 of Cdc42 and Rac1 is critical for specific recognition by Don1, Its1 and Cdc24, Hot1 is insensitive to changes at this position. Instead, Hot1 relies on a different set of amino acids to bind its specific target Cdc42. We could demonstrate that this unusual mechanism to discriminate between different Rho-type GTPases is also used by the mammalian orthologue of Hot1, TUBA1. These data allowed us to generate a chimeric Cdc42/Rac1 GTPase which can be activated by both Cdc42- and Rac1-specific Rho-GEFs with comparable efficiency. Importantly, such a chimeric GTPase was able to complement the morphological phenotypes of Cdc42 and Rac1 deletion mutants *in vivo*.

161. Moisture dependencies of *P. Rubens* on a porous substrate. K.A. van Laarhoven[1], F.J.J. Segers[2], J. Dijksterhuis[2], H.P. Huinink[1], O.C.G. Adan[1]. 1) Eindhoven University of Technology, Eindhoven, Netherlands; 2) CBS - KNAW, Utrecht, Netherlands.

Fungal growth indoors can lead to both disfigurement of the dwelling and medical problems such as asthma. It is generally accepted that the primary cause for mould growth is the presence of moisture. Strategies to prevent fungal growth are therefore often based on controlling indoor humidity. Still, mould is often encountered in ventilated buildings that are considered to be relatively dry. Preliminary experiments showed that fungi can survive on porous materials due to short intervals of favorable circumstances; even when - on average - conditions for growth are not met. This suggests that the interactions between porous materials and the fluctuating indoor humidity play an important role in a colony's survival. We study this interplay between indoor climate, substrate water household and fungal growth. A property of water that is crucial for fungal growth is water activity (a_w). This property determines a fungus's ability to take up water. The effect of a_w on fungal growth has been determined in the past by extensive growth experiments on agar, and many previous studies of growth on building materials take this parameter into account. Up till now, however, little attention has been paid to the water content (q) of a substrate, which represents the amount of water that is physically present in a system. In most porous materials, even when aw is relatively high, only little water is present. We suspect therefore that growth on porous substrates is limited by water content (whereas on agar, q is always close to 100% and will therefore be of little concern). We performed growth experiments with *P. rubens* inoculated on gypsum while separately controlling q and a_w. Video microscopy was used to monitor the germination and subsequent growth of hyphae. The early development of the fungus was

then quantified by determining parameters such as germination time and growth speed from the movies. The experiments show that the germination rate, growth speed and growth density of *P. rubens* on gypsum increase with *q* while aw is constant, and increase with a_w while *q* is constant. We conclude from this that *q* and aw have separate effects on growth on porous substrates. An explanation for the effect of *q* could be that it limits a fungus's access to both water and nutrients. Follow up research will focus on modeling and explaining these effects.

162. Localization of Ga proteins during germination in the filamentous fungus, Neurospora crassa. Ilva Esther Cabrera[1], Carla Eaton[2], Jacqueline Servin[1], Katherine Borkovich[1]. 1) Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA; 2) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.

Heterotrimeric G protein signaling is essential for normal hyphal growth in the filamentous fungus Neurospora crassa. We have previously demonstrated that the non-receptor guanine nucleotide exchange factor RIC8 acts upstream of the Ga proteins GNA-1 and GNA-3 to regulate hyphal extension. Germination assays revealed essential roles for RIC8 and GNA-3 during this crucial developmental process. Localization of the three Ga proteins during conidial germination was probed through analysis of cells expressing fluorescently tagged proteins. Functional TagRFP fusions of each of the three Ga subunits were constructed through insertion of TagRFP in a conserved loop region of the Ga subunits. The results demonstrated that GNA-1 localizes to the plasma membrane and vacuoles, and also to septa throughout conidial germination. GNA-2 localizes to both the plasma membrane and vacuoles during early germination, but is then found in vacuoles later during hyphal outgrowth. Interestingly, in addition to y plasma membrane and vacuolar localization, GNA-3 was found in distinct patches on the plasma membrane of the original conidium during early germination. This distinct localization of GNA-3 supports the hypothesis that GNA-3 is needed for proper conidial germination, and this specific localization may be required for development. Further investigation is under way to determine the consequence of this localization. Colocalization of RIC8-GFP with GNA-1-TagRFP or GNA-3-TagRFP was not detected in cells expressing two fluorescent proteins. This finding suggests that their interaction may be transient not able to be captured via this method. A more sensitive microscopic approach is being implemented to better test for colocalization.

163. Deciphering the roles of the secretory pathway key regulators YPT-1 and SEC-4 in the filamentous fungus *Neurospora crassa*. E. Sanchez, M. Riquelme. Center for Scientific Research and Higher Education of Ensenada (CICESE). Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 28860, Ensenada-B.C.-Mexico.

The transport of proteins through different compartments of the secretory pathway is mediated by vesicles. It is well known that vesicular trafficking is regulated by Rab GTPases, which in their active state interact with the membrane of the vesicles. Subsequently, through protein-protein interactions, they coordinately associate with factors involved in transport and/or tethering to the receptor organelle. In contrast to other eukaryotic model systems, most filamentous fungi contain a Spitzenkörper (Spk), which is a multi-vesicular complex found at the hyphal apex to which cargo-carrying vesicles arrive before being redirected to specific cell sites. The exact regulatory mechanisms utilized by the hyphae to ensure the directionality of the secretory vesicles that reach the Spk are still unknown. Hence, we have analyzed the *N. crassa* Rab-GTPases YPT-1 and SEC-4, key regulators of the secretory pathway rather well characterized in *S. cerevisiae*. YPT-1 regulates ER-Golgi and late endosome-Golgi traffic steps, while SEC-4 regulates post-Golgi vesicle traffic en route to the plasma membrane. Laser scanning confocal microscopy of strains expressing fluorescently tagged versions of the proteins revealed that YPT-1 localizes at the Spk microvesicular core and at cytoplasmic pleomorphic punctate structures, suggesting its participation in different traffic steps. YPT-1 accumulation at the Spk might suggest its function in mediating the traffic of vesicles from early endosomes as a recycling process. The pleomorphic structures could correspond to late Golgi equivalents. The localization of SEC-4 at the Spk, suggests the participation of this Rab in late traffic steps of Golgi-derived vesicles previous to exocytic events. The relative distribution of both Rabs compared to the molecular motor MYO-2 (presumably involved in secretory vesicle transport), the long coiled-coil protein USO-1 (tethering factor), the secreted protein INV-1, and proteins involved in cell wall biosynthesis is being analyzed and will provide better clues on the nature of the identified compartments.

164. Functional characterization of CBM18 proteins, an expanded family of chitin binding genes in the *Batrachochytrium dendrobatidis* genome. Peng Liu, Jason Stajich. Plant Pathology & Microbiology, Univ California, Riverside, Riverside, CA.

Batrachochytrium dendrobatidis (Bd) is the causative agent of chytridiomycosis, one of the major causes of worldwide decline in amphibian populations. Little is known about the molecular mechanisms of its pathogenicity. Our previous work[1] from the initial analysis of the Bd genome revealed a unique expansion 18 copies of the carbohydrate-binding module family 18 (CBM18), specific to Bd, and evolving under positive directional selection. CBM18 is predicted to be a sub-class of chitin recognition domains. Our hypothesis is that some of these copies of CBM18 can bind chitin, a major component of fungal cell walls, in vitro. In order to investigate CBM18's intracellular localization, four CBM18 genes, representing tyrosinase-like, deacetylase-like and lectin-like groups, were cloned into a yeast GFP expression vector. Only two genes from lectin-like group fused with GFP, showing cell boundary localization. Furthermore, intracellular signals were observed on both GFP fusion proteins. According to the TargetP database, both proteins are predicted to have the secretion signal peptide. When co-stained with FM4-64, a dye to label vacuole membranes, the FM4-64 and GFP signals were mutually exclusive, indicating that the GFP fusion proteins were not destined for degradation. Expression of the proteins from the pHIL-S1 vector in the Pichia system will enable purification and characterization of binding properties of these molecules and affinity for chitin and other substrates. 1. Abramyan and Stajich, mBio 2012; 3(3): e00150-12.

165. The exocyst complex is necessary for secretion of effector proteins during plant infection by *Magnaporthe oryzae*. Yogesh K. Gupta[1], Martha Giraldo[2], Yasin Dagdas[1], Barbara Valent[2], Nicholas J. Talbot[1]. 1) School of Biosciences, University of Exeter, EX4 4QD, UK; 2) Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA.

Magnaporthe oryzae is a devastating plant pathogenic fungus, which causes blast disease in a broad range of cereals and grasses. A specialized infection structure called the appressorium breaches the leaf cuticle and subsequently the fungus colonizes host epidermal cells. Colonization of host tissue is facilitated by small secreted proteins called effectors, that suppress plant immunity responses and may also mediate invasive growth. Some of these effectors have been shown to localize at the appressorium pore prior to plant infection, at the tips of primary invasive hyphae and in a specialized plant-derived, membrane-rich structure called the Biotrophic Interfacial Complex (BIC). However the underlying mechanism controlling polarized secretion is not well defined in *M. oryzae*. The exocyst is an octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that appears to be evolutionary conserved in fungi and to play a crucial role in vesicle tethering to the plasma-membrane. The exocyst plays an important role in polarized exocytosis and interacts with various signaling pathways at the apex of fungal cells. We are currently characterizing components of exocyst complex during infection related development of *M. oryzae*. We have shown that the exocyst localizes to hyphal tips as in other fungi during hyphal growth in culture. Interestingly, exocyst components also localize around the appressorium pore, which suggests the pore is an active site for secretion at the point of plant infection. We have recently shown that organization of the appressorium pore requires a hetero polymeric septin network and we show

here that localization of the exocyst at the appressorium pore is septin dependent. The exocyst is furthermore involved in secretion of symplastic (host cell-delivered) effectors but not apoplastic effectors. Targeted gene deletion of exocyst components Exo70 and Sec5 causes significant virulence defects because of impaired secretion. We will present new information on the role of the exocyst during invasive growth of *M. oryzae*.

166. Functional analysis of protein ubiquitination in the rice blast fungus *Magnaporthe oryzae*. Yeonyee Oh, Hayde Eng, William Franck, David Muddiman, Ralph Dean. Dept Plant Pathology, NCSU, Raleigh, NC.

Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, *Magnaporthe oryzae*. Protein ubiquitination, which is highly selective, regulates many important biological processes including cellular differentiation and pathogenesis in fungi. Gene expression analysis revealed that a number of genes associated with protein ubiquitination were developmentally regulated during spore germination and appressorium formation. We identified an E3 ubiquitin ligase, MGG_13065 is induced during appressorium formation. MGG_13065 is homologous to fungal F-box proteins including *Saccharomyces cerevisiae* Grr1, a component of the Skp1-Cullin-F-box protein (SCFGrr1) E3 ligase complex. Targeted gene deletion of MGG_13065 resulted in pleiotropic effects on *M. oryzae* including abnormal conidia morphology, reduced growth and sporulation, reduced germination and appressorium formation and the inability to cause disease. Our study suggests that MGG_13065 mediated ubiquitination of target proteins plays an important role in nutrient assimilation, morphogenesis and pathogenicity of *M. oryzae*.

167. The role of autophagy in *Cryphonectria hypovirus 1* (CHV1) infection in *Cryphonectria parasitica*. M. Rossi, M. Vallino, S. Abba', M. Turina. Institute of Plant Virology, National Research Council (CNR), Torino, Italy.

The interaction between *Cryphonectria parasitica*, the causal agent of chestnut blight, and *Cryphonectria hypovirus* 1 (CHV1) results in fungal hypovirulence associated with alterations of fungal development, reduced sporulation and pigmentation, accumulation of cytosolic vesicles. The role of these vesicles is to support CHV1 maintenance and replication, but the origin of these compartments is still under debate. Due to the phylogenetic proximity between CHV1 and poliovirus, which induces autophagosome proliferation in infected cells, we decided to explore the involvement of autophagy in vesicle accumulation and virus replication in CHV1-infected mycelium. We are studying the autophagy dynamic in CHV1-infected *Cryphonectria* expressing GFP-CpAtg8. Atg8 is the fungal orthologue of the mammalian LC3, an essential protein for autophagosome formation which is considered a reliable autophagosome marker. In CHV1-free hyphae, GFP-CpAtg8 distribution was mostly cytosolic, but in presence of CHV1 we observed a punctate distribution of fluorescence which is compatible with the binding of GFP-CpAtg8 with autophagosome membranes. The induction of autophagy is also supported by the observed increase of accumulation of GFP-CpAtg8 in presence of CHV1 compared with virus-free mycelium which could be due to an activation of gene transcription and/or to protein stabilization. Overall our results seem to confirm the activation of autophagy by CHV1. We are now testing through various approaches if CHV1 is able to induce autophagosomes proliferation to support its own replication or if this is an effect of fungal defense against hypovirus infection.

168. *Neurospora crassa* protein arginine methyl transferases are involved in growth and development and interact with the NDR kinase COT1. D. Feldman, C. Ziv, M. Efrat, O. Yarden. Dept of Plant Pathology and Microbiology, Faculty of Agricutlure, The Hebrew University of Jerusalem, Rehovot, Israel.

The protein arginine methyltransferaseas (PRMTs) family is conserved from yeast to human, and regulates stability, localization and activity of proteins. We have characterized deletion strains corresponding to genes encoding for PRMT1/3/5 (designated *prm-1, prm-3* and *skb-1*, respectively) in *N. crassa*. Deletion of PRMT-encoding genes conferred reduced growth rates and altered Arg-methylated protein profiles (as determined immunologically). D*prm-1* exhibited reduced hyphal elongation rates (70% of wild type) and increased susceptibility to the ergosterol biosynthesis inhibitor voriconazole. In D*prm-3*, distances between branches were significantly longer than the wild type, suggesting this gene is required for proper regulation of hyphal branching. Deletion of *skb-1* resulted in hyper conidiation (2-fold of the wt) and increased tolerance to the chitin synthase inhibitor polyoxin D. Inactivation of two PRMTs responsible for asymmetric dimethylation (D*prm-1*;D*prm-3*) conferred changes in both asymmetric as well as symmetric protein methylation profiles, suggesting either common substrates or cross-regulation of different PRMTs. Taken together, all *N. crassa* PRMTs are involved in fungal growth, hyphal cell integrity and affect asexual (but not sexual) reproduction. The PRMTs in *N. crassa* apparently share cellular pathways which were previously reported to be regulated by the NDR (Nuclear DBF2-related) kinase COT1, whose dysfunction leads to a pleiotropic change in hyphal morphology. Using co-immunpercipitation experiments, we have shown that SKB1 and COT1 can physically interact. To date, two isoforms of COT1 (67 and 73KDa) have been identified and studied. We have now identified a third, 70kDa, isoform of COT1, whose abundance was increased in a D*skb-1* background. This isoform, as well as the two others, are Arg-methylated, as determined on the basis of immunological detection and results indicate that the methylation observed involves the activity of more than one PRMT enzyme. The fact that environmental suppression of the *cot-1* phenotype is more pronounced in *prm-3* and *skb-1* backgrounds links these PRMTs to the environmental response associated with COT1 function. Based on the highly conserved structure of the PRMTs and the NDR kinases in eukaryotes, it is likely that these proteins undergo similar interactions in other organisms.

169. Role of *tea1* and *tea4* homologs in cell morphogenesis in *Ustilago maydis*. Flora Banuett, Woraratanadharm Tad, Lu Ching-yu, Valinluck Michael. Biological Sciences, California State University, Long Beach, CA.

We are interested in understanding the molecular mechanisms that govern cell morphogenesis in *Ustilago maydis*. This fungus is a member of the Basidiomycota and exhibits a yeast-like and a filamentous form. The latter induces tumor formation in maize (*Zea mays*) and teosinte (*Zea mays* subsp. *parviglumis* and subsp. *mexicana*). We used a genetic screen to isolate mutants with altered cell morphology and defects in nuclear position. One of the mutants led to identification of *tea4*. Tea4 was first identified in *Schizosaccharomyces pombe*, where it interacts with Tea1 and other proteins that determine the axis of polarized growth. Tea4 recruits a formin (For3), which nucleates actin cables towards the site of growth, and thus, polarizes secretion (Martin et al., 2005). Tea1 and Tea4 have been characterized in *Aspergillus nidulans* and *Magnaporthe oryzae* (Higashitsuji et al., 2009; Patkar et al., 2010; Takeshita et al., 2008; Yasin et al., 2012). Here we report the characterization for the first time of the Tea4 and Tea1 homologs in the Basidiomycota. The *U. maydis tea4* ORF has coding information for a protein of 1684 amino acid residues that contains a Src homology (SH3) domain, a RAS-associating domain, a phosphatase binding domain, a putative NLS, and a conserved domain of unknown function. All Tea4 homologs in the Basidiomycota contain a RA domain. This domain is absent in Tea4 homologs in the Ascomycota, suggesting that Tea4 performs additional functions in the Basidiomycota. We also identified the Um*tea1* homolog, which codes for a putative protein of 1698 amino acid residues. It contains three Kelch repeats. The Tea1 homologs in the Ascomycota and Basidiomycota contain variable numbers of Kelch repeats. The Kelch repeat is a protein domain involved in protein-protein interactions. The *tea1* gene was first identified in *S. pombe* and is a key determinant of directionality of polarized growth (Mata and Nurse, 1997). To understand the function of *tea1* and *tea4* in several cellular processes in *U. maydis*, we generated null mutations. We demonstrate that *tea4* and *tea1* are necessary for the axis of polarized growth, cell polarity, normal septum positioning, and organization of the microbutubule cytoskeleton. We also determined the subcellular localization of Tea1::GFP and Tea4::GFP in the yeast-like and filamentous forms.

170. Sex determination directs uniparental mitochondrial inheritance in *Phycomyces blakesleeanus*. Viplendra P.S. Shakya, Alexander Idnurm. School of Biological Sciences, University of Missouri-Kansas City, MO.

Uniparental inheritance (UPI) of mitochondria is common among eukaryotes. Various mechanisms have been suggested for UPI, but the underlying molecular basis is yet to be fully explained. We used a series of genetic crosses to establish that the *sexM* and *sexP* genes in the mating type locus control the UPI of mitochondria in the Mucoromycotina fungus *Phycomyces blakesleeanus*. Inheritance is from the (+) sex type, and is associated with degradation of the mitochondrial DNA from the (-) parent in the developing zygospore. Hence, the UPI of mitochondria in *Phycomyces* shows that this process can be directly controlled by genes that determine sex identity, independent of cell size or the complexity of the genetic composition of a sex chromosome.

171. Exploring the role of a highly expressed, secreted tyrosinase in Histoplasma capsulatum mycelia. Christopher F. Villalta[1], Dana Gebhart[2], Anita Sil[1]. 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) AvidBiotics Corporation, South San Francisco, California, United States of America.

The human pathogen *Histoplasma capsulatum* is a dimorphic ascomycete that resides in the soil at ambient temperature as a mycelium. Infection of immunocompetent individuals with *H. capsulatum* occurs when mycelial fragments and associated conidia are inhaled. These fungal cells undergo a conversion to a budding-yeast form in response to mammalian body temperature. We are interested in genes that specify the biological attributes of either the infectious form (mycelia or conidia) or the parasitic form (yeast). Previous work from our lab compared the gene expression profiles of mycelia, conidia, and yeast cells to determine genes that were preferentially expressed in each developmental form. We determined that the *TYR1* gene, which encodes a putative polyphenol oxidase, or "tyrosinase", is highly differentially expressed in the mycelial form of *H. capsulatum*. Notably, the *H. capsulatum* genome contains seven tyrosinases, all of which are more highly expressed in mycelia and conidia compared to yeast. These enzymes contain a conserved tyrosinase domain, but their function in pathogenic fungi has not been investigated. Our expression data suggest that tyrosinases play a specific role in the biology of *H. capsulatum* filaments and spores. Strains that either lack *TYR1* or express deregulated *TYR1* display altered growth properties during the mycelial phase. Interestingly, our preliminary results indicate that Tyr1 is secreted into the media during mycelial-phase growth. We are currently investigating whether Tyr1 affects mycelial growth by modifying a cell-surface or secreted molecule. Additionally, we are determining if Tyr1 is important in the production of infectious spores.

172. Hypobranching induced by both anti-oxidants and ROS control gene knockouts in Neurospora crassa. Michael K. Watters, Jacob Yablonowski, Tayler Grashel, Hamzah Abduljabar. Dept Biol, Valparaiso Univ, Valparaiso, IN.

Wild-type Neurospora grows with the same branch density (statistical distribution of physical distances between branch points along a growing hypha) at a wide range of incubation temperatures. Previous work highlighted the impact of reactive oxygen species (ROS) control on branch density. Here we report the branching effects of selected ROS control gene knockout mutants; the impact of exogenously added anti-oxidants. In all ROS control mutants tested, growth was shown to branch tighter when grown at higher temperatures and looser when grown at lower temperatures. The branch density displayed by the ROS mutants at low temperature is measurably hypobranched. In tests on wild type Neurospora, added Ascorbic Acid and Glutathione produced unusual branching patterns. Hypha exposed to Ascorbic Acid or Glutathione display a distribution of branching with two distinct maxima. They show an increase in both very closely spaced branching as well as an increase in more distantly spaced branching. At lower doses however, hypobranching, again, is observed with average branch density being linearly related to the dose of added anti-oxidants. We also report on the interaction between ROS mutants and added anti-oxidants.

173. Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites. Diego Delgado-Álvarez[1], S. Seiler[2], S. Bartnicki-García[1], R. Mouriño-Pérez[1]. 1) CICESE, Ensenada, Mexico; 2) Georg August University, Göttingen, Germany.

The machinery responsible for cytokinesis and septum formation is well conserved among eukaryotes. Its main components are actin and myosins, which form a contractile actomyosin ring (CAR). The constriction of the CAR is coupled to the centripetal growth of plasma membrane and deposition of cell wall. In filamentous fungi, such as Neurospora crassa, cytokinesis in vegetative hyphae is incomplete and results in the formation of a centrally perforated septum. We have followed the molecular events that precede formation of septa and constructed a timeline that shows that a tangle of actin filaments is the first element to conspicuously localize at future septation sites. We named this structure the SAT for septal actin tangle. SAT formation seems to be the first event in CAR formation and precedes the recruitment of the anillin Bud-4, and the formin Bni-1, known to be essential for septum formation. During the transition from SAT to CAR, tropomyosin is recruited to the actin cables. . Constriction of the CAR occurs simultaneously with membrane internalization and synthesis of the septal cell wall.

174. Characterization of the *Neurospora crassa* STRIPAK complex. Anne Dettmann[1], Yvonne Heilig[1], Sarah Ludwig[1], Julia Illgen[2], Andre Fleissner[2], Stephan Seiler[1]. 1) Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany; 2) Biozentrum, Technische Universität Braunschweig,Germany.

The majority of fungi grow by polar tip extension, branching and intercellular fusion to generate a supra-cellular, syncitial mycelium. This hyphal network formation increases the fitness of the organisms and is central to the organization and function of the fungal colony. Multiple mutants deficient in hyphal fusion and/or intercellular signaling were characterized in *Neurospora crassa*, the currently best understood model for interhyphal signaling. Among them are components of the two MAK1 and MAK2 MAP kinase cascades and a cell fusion-specific phosphatase 2A termed the STRIPAK complex. While the MAK2 cascade is central for signaling through oscillatory recruitment of the MAK2 module to opposing tips of communicating cells, the MAK1 cell wall integrity pathway is assumed to play a critical role in the cell wall rearrangement after the physical contact of the two partner cells. The mechanistic function of the STRIPAK complex and the functional relationship of the three modules is not resolved. By a combination of genetic, biochemical and life cell imaging techniques, we present the characterization of the STRIPAK complex of *N. crassa* that consists of HAM2/STRIP, HAM3/striatin, HAM4/SLMAP, MOB3/phocein, PPG1/PP2AC and PP2AA. We further describe that the fungal STRIPAK complex localizes to the nuclear envelope and regulates the nuclear accumulation of the MAP kinase MAK1 in a MAK2-dependent manner.

175. Does the CENP-T-W-S-X tetramer link centromeres to kinetochores? Jonathan Galazka, Mu Feng, Michael Freitag. Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

In vertebrates, the centromeric proteins, CENP-T, -W, -S and -X, form a tetramer (CENP-T-W-S-X) *in vitro* that binds DNA [1]. Furthermore, the unstructured N-terminus of CENP-T interacts with the Ndc80 complex at kinetochores [2]. This suggests that CENP-T-W-S-X has a central role in linking centromeric DNA to kinetochores. Despite the appeal of this model, there is no evidence that this complex forms *in vivo*, no information of the DNA sequences it may bind at centromeres and little understanding of how it interacts with canonical nucleosomes. CENP-T, -W, -S, and -X are conserved in fungi, including *Neurospora* [1-3]. *Neurospora* is an attractive model in which to understand the function of the CENP-T-W-S-X complex as its centromeric

DNA is nearly completely assembled, allowing ChIP-seq reads to be mapped unambiguously [4]. Here, we report on our investigations of the *Neurospora* CENP-T-W-S-X complex, including its interactions with centromeric DNA and canonical centromeric nucleosomes.

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[3] Smith, K. M. et al. 2012. Centromeres of filamentous fungi. Chrom. Res. 20, 635-656.
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176. Proper actin ring formation and septum constriction requires coordination of SIN and MOR pathways through the germinal centre kinase MST1. Yvonne Heilig, Anne Dettmann, Stephan Seiler. Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany.

The highly conserved nuclear Dbf2p-related (NDR) kinases control polar morphogenesis and cell proliferation. In fungi, NDR kinases function as effectors of the morphogenesis (MOR) and septation initiation (SIN) networks and are activated by germinal centre (GC) kinases. The *Neurospora crassa* SIN kinases SID1 and DBF2 are essential for septum formation. In contrast, the MOR kinases POD6 and COT1 promote apical tip growth and function as negative regulators of septation. We identified a third GC kinase MST1 that functions as promiscuous enzyme, activating DBF2 and COT1. As typical for SIN components, MST1 localized to spindle pole bodies and constricting septa. Moreover, D*mst-1* displayed synthetic interactions with *sin*, but not *mor* mutants, placing MST1 in parallel to the central SIN kinase cascade CDC7-SID1-DBF2. Consistent with these genetic data, we determined that the two GC kinases MST1 and SID1 are regulated by CDC7 in an opposite manner. Lifeact- and formin-GFP reporter constructs revealed the formation of aberrant cortical actin rings in D*mst-1*, which resulted in mispositioned septa and irregular spirals in the mutant. In summary, our data identify an antagonistic relationship between the SIN and MOR during septum formation that is, at least in parts, coordinated through the GC kinase MST1.

177. Regulatation of the BUD3-BUD4 landmark complex by the NDR kinases DBF2 and COT1 during septum formation in *Neurospora crassa*. Yvonne Heilig, Stephan Seiler. Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany.

Cytokinesis is essential for cell proliferation, yet the mechanisms for determining the site of cell division are poorly understood. Our data indicate that the anillin BUD4 marks septum placement by organizing the RHO4-BUD3-BUD4 GTPase module and that this complex is controlled through two NDR kinase signaling cascades, the septation initiation network (SIN) and the morphogenesis network (MOR). Epistasis analysis of *sin* and *mor* mutants places the SIN upstream of the MOR. DBF2 functions as competitive inhibitor of COT1 by forming hetero-dimers, thereby replacing the COT1 co-activators MOB2A/B. In turn, COT1 functions as negative regulator of septum formation. We demonstrate that COT1, but not DBF2, binds to and phosphorylates BUD3 and BUD4. Mutational analysis of BUD3 identifies Ser798, located within an amphiphatic helix of BUD3 that is phosphorylated by COT1. Localization of this amphiphatic helix at septa is only possible in its nonphosphorylated form. In summary, our data suggest a model, in which the MOR kinase COT1 phosphorylates BUD3 and BUD4 and that this modification inhibits cortical localization and function of the BUD complex. Interference of the SIN with MOR activity at the septum relieves this inhibition and allows initiation of septation.

178. Development of a Protein-Protein Interaction Platform in *Neurospora Crassa*. Shouqiang Ouyang, Katherine Borkovich. Plantn Pathology and Microbiology, University of California, Riverside, Riverside, CA.

The objective of this study is to generate a protein-protein interaction platform for *Neurospora crassa*. We have constructed Dmus-51::nat and Dmus-52::nat strains that also carry the Drid::nat mutation to eliminate RIP. These strains are used as recipients for transformation. Ten genes were solicited as candidates from the N. crassa community, including SAD-1/SAD-2, WC-1/WC-2, FRQ/FRH, OS-4/ RRG-1and GNB-1/GNG-1. We construct vectors for each protein by amplifying the ORFs from wild type *N. crassa* genomic DNA using gene-specific primers. Protein constructs are expressed with a V5-GFP or S-tag-RFP tag from the pan-2 or inl locus, respectively in *N. crassa*. Protein complexes can be isolated by immunoprecipitation using antibody to the GFP/V5 or RFP/S-tag epitope. Both immunoprecipitation and the overlap localization of fluorescent proteins (GFP and RFP) data will streamline our ability to monitor protein-protein interactions and co-localization *in vivo* in *N. crassa*.

179. Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in *Neurospora crassa*. Martin Weichert[1], Ewald Priegnitz[1], Raphael Brandt[1], Thorben Nawrath[2], Stefan Schulz[2], André Fleissner[1]. 1) Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany; 2) Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany.

Sterols are major constituents in the plasma membrane of eukaryotic cells. They modulate the physical properties of the lipid bilayer, e.g. fluidity. By interacting with certain lipids and proteins in the plasma membrane, sterols cluster into microdomains which might act as platforms for many biological functions, such as signal transduction. In the early stages of colony formation in *Neurospora crassa*, germinating spores direct their growth towards each other, establish physical contact, and fuse. Cell-to-cell signaling requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1. Here, we report that mutants affected in the biosynthesis of ergosterol, the major sterol in most fungal species, show distinct defects during germling fusion. Deletion of *erg-2*, which encodes an enzyme mediating the last step in the pathway, strongly impairs both directed growth and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting D*erg-2* germlings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, D*erg-10a* D*erg-10b* germling pairs show D*Prm1*-like deficiencies in plasma membrane merger. By relating the sterol composition and fusion competence of several *erg* mutants, we find that not the absence of ergosterol but the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes D*erg-2*-like deficiencies, an altered double bond arrangement in the sterol ring system causes D*Prm1*-like defects. During sexual development, cell fusion precedes the fertilization of fruiting bodies. Unlike the defects during germling fusion, female and male mating partners of D*erg-2* and D*erg-10a* D*erg-10b* efficiently fuse, suggesting that alterations in the sterol composition specifically impair signaling mechanisms mediating vegetative cell fusion. These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signaling complexes, and plasma membrane fusion.

180. The role of NADPH oxidases in *Neurospora crassa* cell fusion. Nallely Cano-Dominguez[1], Ernestina Casto-Longoria[1], Jesus Aguirre[2]. 1) Departamento de Microbiologia, CICESE, Ensenada, Baja California, Mexico; 2) Departamento de Biologia Celular y Desarrollo. Instituto de Fisiologia Celular UNAM, Mexico City, D.F. Mexico.

Hansberg and Aguirre proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by the action of certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production

of superoxide by transferring electrons from NADPH to oxygen. *Neurospora crassa* contains the NADPH oxidases NOX-1 and NOX-2 and a common regulatory subunit NOR-1. NOX-2 is essential for ascospore germination, while NOX-1 is required for sexual and asexual development, polar growth and cell fusion. NOR-1 is essential for all these NOX functions. We have found that a functional NOR-1::GFP fusion is localized throughout the cytoplasm, enriched at the hyphal tip and sometimes in aggregates. This suggests that the functional NOX complexes are probably not localized at the plasma membrane. Up to now NOX function in fungi has been evaluated in mutants that completely lack NOX proteins. We generated nox-1 alleles that result in NOX-1 proteins carrying substitutions of proline 382 by histidine or cysteine 542 by arginine, which affect NADPH-binding. Equivalent mutations in phagocytic Nox2/gp91phox do not affect protein stability but completely lack oxidase activity. P382H and C542R mutants did not produce sexual fruiting bodies and showed a decreased growth and differentiation of aerial mycelia, without affecting production of conida. These results indicate that sexual development depends on ROS production by NOX-1, whereas during asexual differentiation NOX-1 plays an important role independently of its catalytic activity. Dnox-1, Dnor-1, P382H NOX-1 and C542R NOX-1 mutants were all able to produce some conidial anastomosis tubes (CATs) but they were unable to complete cell-cell fusion. All these mutants are also impaired in vegetative hyphae-hyphae fusion, which might explain the growth defects in Dnox-1 and Dnor-1 strains. CATs production is delayed in the presence of antioxidant N- acetyl cystein (NAC) and Dsod-1 strains show an increase in CATs fusions. The results suggest that some ROS may be implicated in signaling CATs homing and vegetative fusion.

181. DYNAMICS OF THE PROTEINS BUD-2 AND BUD-5 DURING CELL POLARIZATION IN *NEUROSPORA CRASSA*. E. Castro-Longoria, C. Araujo-Palomares, N. Cano-Domínguez. Microbiology Department, CICESE. Carretera Ensenada-Tijuana No 3918 Zona Playitas, C.P. 22860. Ensenada, B.C. México.

Polarized growth in filamentous fungus requires an excellent and precise machinery to select specific sites where multiple protein complexes assemble to ensure the generation of highly polarized hyphae. One of these protein complexes is the Rsr1p/Bud1p-Bud2p-Bud5p module, which, in *Saccharomyces cerevisiae*, has the function of selecting the proper site of budding. However, in filamentous fungi the function of this module is unknown. In this study, we characterized the intracellular localization and dynamics of protein homologues for BUD-2 and BUD-5 in the filamentous fungus *Neurospora crassa*. Preliminary results of *in vivo* confocal microscopy analysis shows that both BUD-2 and BUD-5 display distinct localization patterns in both mature hyphae and germlings. In mature hyphae, BUD-2 localization is confined to the apical cytosol, occupying the core of the Spitzenkörper (Spk), while BUD-5 was observed in the apical region of the cells as a bright spot with higher intensity at the center base adopting a hand fan shape, partially colocalizing with the Spk. In contrast, BUD-2 in germlings was associated with the cell membrane and organized as a cap shape covering the apex of the cells, while BUD-5 localization was observed in three different ways: as a bright spot at the apex of germinating spores, then as a cytosolic crescent-shape in longer germ tubes and finally adopting a similar localization pattern as in mature hyphae. BUD-2 and BUD-5 also display distinct localization patterns during branching and septum formation. BUD-2 participates in septum formation while BUD-5 was only involved during the initiation of lateral branches. The distinct cellular localization patterns of BUD-2 and BUD-5 suggest that although both proteins may be important for cell polarity establishment, they also participate in other morphogenetic processes in *N. crassa*.

182. The role of calcium and calmodulin during cell fusion and colony initiation in *Neurospora crassa*. Chia-Chen Chang, Nick Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH.

Calcium is an ubiquitous signalling molecule which regulates many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, circadian rhythms, and virulence. Transient increases in cytosolic free calcium ($[Ca^{2+}]_c$) act as intracellular signals. As the primary intracellular Ca^{2+} receptor, calmodulin (CaM) converts these Ca^{2+} signals into responses by regulating the activity of numerous target proteins. We have found that both Ca^{2+}-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit a form of cell fusion called conidial anastomosis tube (CAT) fusion that occurs during colony initiation in the fungal model *Neurospora crassa*. GFP labelled CaM localized as dynamic particles associated with the plasma membrane and moved around within the cytoplasm in both germ tubes and CATs. In particular, CaM showed a dynamic accumulation at two growing tips of CATs that exhibit chemoattraction towards each other. CaM also localized at developing septa in germ tubes. The b-tubulin inhibitor, benomyl, reduced the movement of CaM in the cytoplasm. Moreover, the absence of extracellular Ca^{2+} inhibited the recruitment of CaM to CAT tips as well as inhibiting CAT chemoattraction. The deletion of the *myosin-5* (*myo-5*) gene caused the mis-localization of CaM in tips of growing germ tube and CATs. This suggests that the movement of cytoplasmic CaM involves transport along microtubules, and the recruitment of CaM to tips involves myosin-5 along F-actin and is dependent on extracellular Ca^{2+}.

183. Deletion of cAMP phosphodiesterase *pde-2*/*acon-2* gene causes the enhanced osmotic sensitivity in *os-1* and *os-2* mutants of *N. crassa*. C. Kurata, M. Kamei, S. Banno, M. Fujimura. Dept Life Sci, Toyo Univ, Gunma, Japan.

N. crassa has two putative cyclic nucleotide phosphodiesterases, PDE-1 (NCU00237) and PDE-2/ACON-2 (NCU00478). The *pde-2* disruptants showed the normal mycelial growth but lacked the ability to produce conidia, these phenotypes resembled those of the *hah* mutant which has a point mutation in the PKA (protein kinase A) regulatory subunit gene. The phenotypes of double mutants, *pde-2;pkac-1* and *hah;pkac-1* mutants, resembled those of the *pkac-1* mutant which shows slow growth and hyperconidiation. In contrast, hyperconidiation of the adenylyl cyclase *cr-1* mutant was suppressed by the *hah* mutation but not by the *pde-2* mutation. These results indicate that PDE-2 act as a major cAMP phosphodiesterase in cAMP-PKA pathway, its deletion leads to the hyper-activation of this pathway. Any mutants in cAMP-PKA pathway including *pde-2* and *hah* mutants, did not show osmotic sensitivity. However, both *pde-2* and *hah* mutations caused the enhanced osmotic sensitivity in *os-1* (histidine kinase) and *os-2* (MAP kinase) mutants, suggesting of cross-talk between cAMP-PKA pathway and OS-2 MAP kinase pathway.

184. Genetic analysis of GNB-1 and CPC-2 with the G alpha subunits in Heterotrimeric G protein signaling in Neurospora crassa. AMruta Garud. Plant Pathology, UC, Riverside, Riverside, CA.

Heterotrimeric G protein signaling is mediated by Gabg subunits. Neurospora crassa has three Ga subunits (GNA-1, GNA-2 and GNA-3), one Gb (GNB-1) and one Gg (GNG-1). The GNB-1 protein contains seven tryptophan-aspartate (WD) repeats, suggesting it assumes a beta propeller form. Genetic epistasis has been demonstrated between gnb-1 and the three Ga subunit genes. gna-3 is epistatic to gnb-1 for submerged culture conidiation, while gna-1 and gna-2 are epistatic to gnb-1 during aerial conidiation. In contrast, gnb-1 is epistatic to gna-2 and gna-3 during aerial hyphae development. Additional proteins that have a 7-WD repeat structure have been implicated as Gb subunits in other fungi. The Cross Pathway Control (CPC-2) protein has a seven WD repeat structure, and shares 70% similarity to the mammalian protein RACK-1. In Neurospora, CPC-2 was previously shown to play a role in general amino acid control. Genetic epistasis with CPC-2 and the Ga proteins is being studied, using strains lacking cpc-2 and one Ga gene, as well as cpc-2 deletion mutants carrying constitutively activated, GTPase-deficient Ga alleles. It is seen that gna-3 is epistatic to cpc-2 during apical extension, aerial hyphae height and asexual sporulation in submerged cultures. gna-1 and gna-2 demonstrate some functional independence. Yeast two hybrid assays show that CPC-2 interacts with GNA-1 and GNA-3. Additional interactions are being examined using additional in vivo and in vitro methods to validate whether CPC-2 acts

as another Gb subunit in Neurospora.

185. Communication Interference during Cell Fusion in *Neurospora crassa* is controlled by a Region under Balancing Selection in the Heterokaryon Incompatibility Locus *het-c*. Jens Heller, Javier Palma-Guerrero, N. Louise Glass. Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA.

Vegetative hyphal fusion events are associated with establishment of a fungal colony. However, non-self recognition during fusion events is important to prevent hybrids between genetically dissimilar individuals that might spread mycoviruses, debilitated organelles, and others throughout a fungal population. In filamentous fungi, the ability of two individuals to form a productive heterokaryon via hyphal fusion is controlled by specific loci termed *het* loci. Stable heterokaryons will only form if the individuals involved have identical alleles at all *het* loci. Accordingly, heterokaryotic cells formed between strains that differ in allelic specificity at one or more *het* loci are rapidly destroyed (programmed cell death) or strongly inhibited in their growth. In *Neurospora crassa*, three allelic specificity groups were identified for *het-c*, which is one of the eleven genetically identified *het* loci in this species. We observed that strains with different haplotypes at *het-c* not only show heterokaryon incompatibility (HI) after cell fusion, but also show reduced chemotrophic interactions and cell fusion between conidial germlings (communication interference). These data indicate that *N. crassa* germlings can distinguish both self and nonself at a distance, and which presumably involves diffusible ligands. Two regions of the glycine-rich single-pass plasma membrane protein HET-C were shown to be under balancing selection and both have different functions. By analyzing different chimeras of *het-c*, we demonstrate that the HET-C specificity domain (amino acids 194-236; region I), is required for inducing programmed cell death during HI, but does not affect communication interference during germling fusion. In contrast, the second region of *het-c* that is also under balancing selection (amino acids 521-599; region II) is responsible for communication interference during germling fusion. To understand the mechanism underlying communication interference, we are identifying which amino acids in HET-C region II are responsible for this trait. In addition, we are determining the cellular localization of HET-C during germling fusion and whether the HET-C region II is a processed form, resulting in a diffusible peptide that is responsible for communication interference during chemotropic interactions and cell fusion of conidial germlings.

186. The *N. crassa* Bem46 protein: alternative splicing and eisosomal association. Krisztina Kolláth-Leib, Frank Kempken. Department of Botany, Christian-Albrechts University, Kiel, Germany.

The bud emergence (BEM) 46 proteins are evolutionarily conserved members of the a/b-hydrolase super family. The exact function(s) of the protein remain unknown. Vegetative hyphae, perithecia and ascospores of *Neurospora crassa* RNAi and over-expressing transformants develop normally, but hyphal germination from ascospores is impaired. These results indicate a role of BEM46 in maintaining cell type-specific polarity in *N. crassa*. In an attempt to further analyse BEM46 function, alternative splicing was observed in the bem46 RNAi line. We present evidence that alternative splice products impair ascospore germination. The BEM46 protein is localized in the perinuclear endoplasmatic reticulum and also forms spots near to the plasma membrane (Mercker et al. 2009). The use of Lifeact-TagRFP (Lichius & Read pers. comm.) and Bem46-eGFP in heterokaryons of *N. crassa* indicated that the Bem46 protein is not interacting with actin. Likewise, the use of the lipid raft-stainer TexasRedTM showed no co-localization with Bem46-eGFP. We analyzed the potential co-localization of Bem46 with the eisosomal protein LSP1. To that end we cloned the corresponding *N. crassa* ortholog of *lsp1* and fused it to RFP. Indeed we were able to demonstrate a co-localization of LSP1 and BEM46. A yeast two-hybrid approach was undertaken using a previously established *N. crassa* two-hybrid library (Seiler pers. comm.). We identified one interacting protein, the anthranylate synthase component II (Walker & DeMoss 1986). Further investigation showed that the BEM46 protein is likely to interact with the F domain of that protein, which is a N-(5'-phosphoribosyl) anthranylate isomerase. The interaction was confirmed *in vivo* by employing bimolecular fluorescence complementation assays.

References:

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187. The alternative oxidase induction pathway is involved in senescence associated with over-replication of a mitochondrial plasmid in *Neurospora crassa*. Nicolette Dutken, Jonathon Gutzeit, Maze Ndonwi, John Kennell. Biology, Saint Louis University, St Louis, MO.

Senescence in *Neurospora crassa* is caused by dysfunctional mitochondria, which is most often due to the effects of mitochondrial plasmids. Variant forms of the Mauriceville plasmid cause senescence by integrating into the mitochondrial genome or by over-replicating, disrupting essential mitochondrial genes or their synthesis. Genetic analysis of plasmid-containing strains that escape senescence indicate that two nuclear mutations are required for longevity. One of the mutations associated with a long lived (LL) strain involves the regulation of Alternative Oxidase (AOX). AOX is induced by mitochondrial dysfunction and is among several nuclear encoded genes involved in mitochondrial function and/or biogenesis that are upregulated during senescence. A model of senescence is proposed in which dysfunctional mitochondria stimulate mitochondrial biogenesis resulting in an accumulation of defective mitochondria. Here we show that the LL strain fails to induce AOX due to a mutation in *aod-2* that encodes a zinc cluster transcriptional regulator of the structural alternative oxidase gene, *aod-1*. Surprisingly, a functional AOX is not required for senescence. This implies that other genes controlled by the AOX induction pathway play a critical role in mitochondrial function in *N. crassa*. Homologs of AOD2 in other fungal species have been shown to regulate gluconeogenesis as well as genes involved in mitochondrial function including subunits of the electron transport chain. Mutations in the AOX induction pathway are not sufficient to overcome plasmid induced senescence and a second nuclear mutation is required. This mutation interferes with the integrative form of senescence and is hypothesized to be associated with either mitochondrial recombination or the selection of mitochondrial rearrangements. The studies of senescence in *N. crassa* provide insights into how fungi respond to mitochondrial damage.

188. Relationship among mutagen sensitivity, senescence and mitochondrial morphology in the *ultraviolet sensitive-5* mutant of *Neurospora crassa*. Kiminori Kurashima, Michael Chae, Hirokazu Inoue, Shin Hatakeyama, Shuuitsu Tanaka. Laboratory of Genetics, Saitama Univercity, Saitama, Japan.

The *uvs-5* mutant of *Neurospora crassa* had been isolated that showed high sensitivity to mutagens (Schroeder, 1970 Mol. Gen. Genet. 107:291-304). This mutant also has phenotypes such as senescent, *i.e.* shortened life span, and progressive accumulation of mitochondrial DNA deletions (Hausner *et al.*, 1995 Fungal Genet. Newsl. 42A: 59). These phenotypes were quite similar to the *mus-10* mutant that we reported previously (Kato *et al*., 2010 Genetics 185:1257-1269). Further, *mus-10* and aged *uvs-5* strains showed fragmented mitochondrial feature although tubular shape was observed in wild type strain. Since we found that the *uvs-5* mutation had been mapped very closely to *fzo1*, which encoded homologue of dynamin-like GTPase mitofusin, the sequence of the *fzo1* gene in the *uvs-5* mutant was determined. A single mutation was found as a deducing amino acid substitution of Gln to Arg in the 386th position locating in the conserved GTPase domain. Forced expression of wild-type FZO1 in the *uvs-5* strain suppressed the defect in mitochondrial morphology and the mutagen sensitivity, but did not in the case of expressing mutated FZO1. Moreover, introduction of this mutation into the endogenous *fzo1* gene of the wild-type strain resulted in showing phenotypes of the *uvs-5* mutant. Thus, we concluded that the responsible gene of *uvs-5*

is *fzo1*. Attempting to disruption the *fzo1* gene was failed, so the *fzo1* gene were suggested to be essential for viability as showing in almost all eukaryotes except for yeast. Therefore the mutation of *uvs-5* may be a useful model for studying the relation between mitochondrial fusion and early senescence in higher eukaryotes.

189. Localization of EGL-1 and EGL-2, two GPI anchored cell wall b (1-3) endoglucanases, at hyphal apices and septa, and in interconidial septa in *Neurospora crassa*. Leonora Martinez, Meritxell Riquelme. Microbiology Department, Center for Scientific Research and Higher Education at Ensenada, Baja California.

The unitary model of cell wall growth suggests that the polarized extension of hyphae in filamentous fungi results from the coordinated synthesis and discharge of new cell wall polymers, the action of hydrolytic enzymes that provide plasticity to the wall and turgor pressure to drive cell expansion. Currently, there is limited information on enzymes capable of hydrolyzing cell wall polymers and that could be contributing to cell wall remodeling. EGL-1 and EGL-2 are putative b (1-3) endoglucanases in *Neurospora crassa*, with potential binding sites for a glucosyl phosphatidylinositol group (GPI), which would allow them to get anchored into the plasma membrane. To investigate whether these proteins participate in key morphogenetic events during the development of N. crassa, EGL-1 and EGL-2 were labeled with the green fluorescent protein (GFP). For *egl-1*, the *gfp* gene was inserted within the *egl-1* encoding sequence, just after the signal peptide sequence. For *egl-2*, the insertion took place right before the GPI-binding site. Both endoglucanases were localized in the hyphal apical plasma membrane and in septa, however, EGL-2-GFP was strongly and more definite localized at the apical dome and EGL-1-GFP showed less intensity with increasing fluorescence from the subapex to the tip. EGL-1-GFP was mostly found at hyphal septa and interconidial septa and EGL-2-GFP was faintly present in a few old septa. Our results suggest that lytic activity of enzymes, such as the endoglucanases EGL-1 and EGL-2 in *N. crassa*, is present in critical areas during vegetative morphogenesis, where these enzymes probably play a role in cell wall remodeling, as postulated by the unitary model of cell wall growth.

190. Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor. Alexander V. Michkov, Katherine A. Borkovich. Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

Heterotrimeric G proteins consist of alpha, beta and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) by the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. GDP/GTP exchange is facilitated by both cell surface G protein coupled receptors and cytosolic guanine nucleotide exchange factors (GEFs), such as RIC8. *Neurospora crassa* has three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1), and one G gamma (GNG-1). Interestingly, mutants lacking *gnb-1* or the cytosolic GEF *ric8* exhibit some defects in common with the *gna-1* deletion mutant, which may be explained by the reduced GNA-1 protein levels observed in these mutants. Previous studies in our laboratory showed that levels of *gna-1* mRNA are similar in wild type and mutants lacking *gnb-1* or *ric8*, consistent with a post-transcriptional mechanism. Using genetic and biochemical approaches, this study investigated the mechanism underlying regulation of GNA-1 stability in regards to GTP/GDP bound state and amount of protein (normal or overexpressed). The results demonstrate that levels of GNA-1 protein are not visibly reduced over 36 hours in a wild-type background after halting translation using cycloheximide, suggesting GNA-1 is very stable in wild type. To check stability of GDP or GTP bound GNA-1 in different backgrounds, we transformed mutants lacking the *gna-1* gene and *gnb-1* or *ric8* with a wild type (*gna-1*^{WT}) or constitutively active, GTPase-deficient *gna-1* allele (*gna-1*^{Q204L}). Overexpressing *gna-1*^{WT} (GDP bound) in a wild-type background increased the level of GNA-1 protein ~ 3 fold, while overexpression in a *gnb-1* mutant gave a nominal increase (~ 1.6x). Overexpressing *gna-1*^{Q204L} (GTP bound) in the *Dgnb-1* or *Dric8* backgrounds led to ~ 2 fold higher levels of GNA-1 compared to wild type. In summary, GNA-1 is very stable in wild type, but stability decreases dramatically in *gnb-1* and *ric8* deletion mutants. The GTP-bound G alpha protein is more stable in a *gnb-1* mutant background than GDP-bound GNA-1 protein.

191. Functional analysis the *Saccharomyces cerevisiae* Ste20, Cla4 homologue in *Neurospora crassa*. Yuhei Nogami, Makoto Fujimura, Akihiko Ichiishi. Faculty of Life Sciences, Toyo University, ORA-GUN, GUNMA, Japan.

Signal transduction pathways are important for a variety of features of fungal development. Small GTPases of Rho family act as molecular switches regulating cell signalling, cytoskeletal organization and vesicle trafficking in eukaryotic cells. The Rho family GTPase Cdc42 was first identified in the yeast *Saccharomyces cerevisiae*, where it is essential for initiation of bud formation and the subsequent switch from apical to isotropic growth. The activation of Cdc42 is catalyzed by Cdc24 guanine nucleotide exchange factors (GEFs), which convert Cdc42 from an inactive GDP-bound form to the active GTP bound form. Bem1 functions as a scaffold connecting Cdc42 with its activator Cdc24. The GTP-bound Cdc42 can activate p21-activated kinase (PAK), Ste20 and Cla4. *Neurospora crassa* has two PAK family kinases Cla4 and Ste20 homologs. We have few knowledge of the function of their PAK kinases in *N. crassa*. In this study, we performed functional analysis of *stk-4* (Ste20 homolog) and *vel* (Cla4 homolog) in *N. crassa*. The *stk-4* deletion mutant showed slow growth than wild type strain, and vel deletion mutant showed more severe growth defects. To determine the subcellular localization and dynamics of STK-4 protein, we constructed GFP-STK-4 fusion constructs. The *gfp* encoding sequence was fused to the 3' end of the *stk-4* open reading frame. We also constructed the GFP-BEM-1 fusion protein. These constructs were introduced into *his-3* locus, and observed using confocal fluorescence microscopy (LSM-510). Both fusion proteins were accumulated at growing hyphal tips and septa. From there results, we consider that STK-4 and BEM-1 are function at the same site.

192. Dissecting the Pathway of Cellulase Secretion in Neurospora crassa. Trevor Starr, Timo Schuerg, Louise Glass. Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

Due to their capacity to secrete large amounts of proteins, particularly hydrolytic enzymes, filamentous fungi are of great interest for high-level protein production in various industries, such as the textile, pharmaceutical, and biofuels industries. Although the basic components of the eukaryotic secretion pathway characterized in yeast and higher organisms are also conserved in filamentous fungi, the highly polarized and compartmentalized growth mode of filamentous fungal hyphae mandates pathways of secretion that are specific to these fungi. While certain aspects of filamentous fungal secretion are under active study, a basic characterization of the entire pathway from start to finish remains to be performed. Such a characterization may provide insights into how filamentous fungi are able to secrete large amounts of enzymes and how these fungi can be engineered to produce even more enzymes in the future. This is particularly of interest to the process of biofuels production, in which the inexpensive production of large amounts of cellulases is a major bottleneck to the efficient and cost-effective production of cellulosic biofuels. In nature the model fungus Neurospora crassa secretes a host of cellulases to allow it to grow on burnt vegetation. The tractability of N. crassa makes it an excellent model to characterize protein secretion in filamentous fungi, particularly the secretion of industrially relevant cellulases. To achieve this goal we are characterizing the cellulase secretion pathway in N. crassa by following the trafficking of fluorescently tagged Endoglucanase 2 (EG-2), a major secreted endocellulase. To determine the compartments through which cellulases traffic we are co-localizing EG-2-GFP with fluorescently-tagged markers of the ER, Golgi, endosomes, and the Spitzenkorper and are assaying the

consequences to EG-2-GFP trafficking of blocks to secretion imposed by pharmacological or mutational insults. Our initial results indicate that EG-2-GFP shows localization to the ER and is mostly absent from the Spitzenkorper, suggesting trafficking through a classical ER to Golgi secretory pathway and terminal secretion along lateral hypahl walls. Additonally, targeted blocks to the secretory pathway indicate a potential role of endosomes in EG-2-GFP trafficking.

193. Towards understanding the endoplasmic reticulum associated degradation process of misfolded glycoproteins in Neurospora crassa. Georgios Tzelepis[1], Hiroto Hirayama[2], Tadashi Suzuki[2], Akira Hosomi[2], Mukesh Dubey[1], Magnus Karlsson[1]. 1) Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 75007, Uppsala, Sweden; 2) Glycometabolome Team, Systems Glycobiology Research Group, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako Saitama 351-0198, Japan.

N-glycosylation is an important post-translational modification of proteins, which occurs in the Endoplasmic Reticulum (ER). These N-linked glycans are reported to play an important role in correct protein folding. Glycoproteins that are unable to fold properly are subjected to destruction by an ER-associated degradation process (ERAD). Degradation of these glycoproteins generates free oligosaccharides (fOs). In animal and plant cells mainly three types of hydrolytic enzymes are involved in the ERAD pathway. First, PNGases which cleave the sugar chain from the protein releasing fOs with N,N'-diacetylchitobiose moieties (fOs-GN2). Secondly, ENGases which catalyse the glycosidic bonds in N,N'-diacetylchitobiose moieties, generating fOs with a single N-acetylglucoseamine at their reducing ends (fOs-GN1), and thirdly, a-mannosidases responsible for trimming the mannose chains before final degradation in lysosomes. The existence of this pathway in filamentous ascomycetes is unknown. In this study we investigate the function of ENGases in N. crassa by analysing the phenotype of deletion strain Dgh18-10 and quantifying the content and type of fOs (fOs-GN1 or fOs-GN2), using dual gradient high performance liquid chromatography. Since cytosolic PNGase is enzymatically inactive in N. crassa, ENGases possibly have a crucial role in the ERAD pathway. We found that deletion of an intracellular ENGase results in severe phenotypic effects. This deletion strain shows significantly slower growth rate in carbon-rich media but grows faster in abiotic stress conditions, indicating a more resistant cell wall. Moreover, the conidiation rate is higher in Dgh18-10 compared to WT. Sexual reproduction is also affected, since no ascospores were observed in Dgh18-10. Additionally, the total amount of extracellular proteins was significantly lower in this deletion strain compared to WT. Finally, this mutation causes repression of three chitin synthase genes in N. crassa. Similar results were also observed in the mycoparasitic ascomycete Trichoderma atroviride. These data may suggest that deletion of cytosolic ENGase leads to accumulation of misfolded glycoproteins in the fungal cytosol, which somehow affects its protein secretion/structure of cell wall. This is the first study of the ERAD pathway in filamentous ascomycetes.

194. *Saccharomyces cerevisiae* spore development and protection against reactive oxygen species. Steve Gorsich, Tricia Stokes, Michelle Steidemann, Kyle Kern. Dept Biol, Central Michigan Univ, Mt Pleasant, MI.

The generation of spores in *S. cerevisiae* is essential for sexual reproduction and survival of the organism. When diploid *S. cerevisiae* cells undergo meiotic division to produce four spores it is important for each spore to not only get a haploid copy of nuclear chromosomes, but also a complete complement of organelles and potentially RNP granules. For instance mitochondria undergo temporally regulated fusion and fission events to assure that mitochondria are represented equally in each of the resulting spores. When this network is not maintained, due to mutations in mitochondrial fission genes (*e.g. dnm1/dnm1*), it has been shown that fewer spores survive and the ones that do survive have reduced respiratory fitness. In addition to mutations affecting spore production we hypothesized that environmental factors could also influence spore development. In the present study, we demonstrated that hydrogen peroxide can phenocopy the mitochondrial fission mutant's phenotypes. Wild-type *S. cerevisiae* exposed to hydrogen peroxide have mitochondrial morphology and distribution defects during spore development, reduced spore viability, and decreased respiratory competency just as seen in *dnm1/dnm1* fission mutants. We next hypothesized that the phenotypes associated with *dnm1/dnm1* mitochondrial fission mutants were caused by increased sensitivity to reactive oxygen species (ROS). To support this we demonstrated that *dnm1/dnm1* mutants have an increase in ROS during spore development. In addition, sporulation defects associated with *dnm1/dnm1* or wild-type cells exposed to hydrogen peroxide were rescued when we overexpressed oxidative stress protection genes. These findings suggest that the ability of *S. cerevisiae* to produce optimal numbers of fit spores is heavily influenced by their ability to protect themselves from exogenous or endogenous ROS.

195. Genetic analysis of the role of peroxisomes in the virulence and survival in *Fusarium graminearum*. K. Min[1], H. Son[1], J. Lee[2], G. J. Choi[3], J.-C. Kim[3], Y.-W. Lee[1]. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; 3) Eco-friendly New Materials Research Group, Research Center for Biobased Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea.

Peroxisomes are single-membrane-bound organelles that are required for diverse biochemical processes, including b-oxidation of fatty acids and detoxification of reactive oxygen species (ROS). In this study, the role of peroxisomes was examined in *Fusarium graminearum* by functional analysis of three genes (*PEX5*, *PEX6*, and *PEX7*) encoding peroxin proteins required for peroxisomal protein import. *PEX5* and *PEX7* deletion mutants failed to localize the fluorescently-tagged peroxisomal targeting signal type 1 (PTS1)- and PTS2-containing proteins to peroxisomes, respectively, whereas the *PEX6* mutant were unable to localize both fluorescent proteins. Deletion of *PEX5* and *PEX6* triggered reduced growth on long chain fatty acids and butyrate, while the *PEX7* deletion mutants utilized fatty acids other than butyrate. Virulence on wheat heads was greatly reduced in the *PEX5* and *PEX6* deletion mutants, because they were impaired in spreading from inoculated florets to the adjacent spikelets through rachis. Disruption of *PEX5* and *PEX6* dropped survivability of aged cells *in planta* and *in vitro* due to the accumulation of ROS followed by necrotic cell death. We suggest that PTS1-type peroxisomal catalases are responsible for ROS scavenging. These results demonstrate the functions of peroxisomes in survival and ROS detoxification of filamentous fungi.

196. roGFP and anti-oxidant defences in the rice blast fungus *Magnaporthe oryzae*. Marketa Samalova, Sarah Gurr, Mark Fricker. Plant Sciences, University of Oxford, Oxford, United Kingdom.

The ascomycete fungus *Magnaporthe oryzae* causes rice blast disease. Germination and development of its infection structure, the appressorium on the host surface is orchestrated by a complex set of signals from within the fungus, and later between the fungus and resistant or susceptible plant that, respectively, either triggers host defence or leads to infection. Host defences involve localised production of reactive oxygen species (ROS), which either kill the pathogen directly or block fungal invasion by oxidative cross-linking of cell wall glycoproteins. By contrast, infection suggests that the invading fungus can tolerate or, indeed, bypass such defences. Here, we report rice blast fungus' capacity to withstand transient oxidative stress during early development. We determine the intrinsic cytoplasmic cell glutathione (GSH) concentration by confocal imaging of monochlorobimane, which becomes fluorescent when conjugated to GSH. The redox poise of the glutathione pool was measured by 4-D confocal excitation ratio imaging of GRX1-roGFP2. We reveal that this fungus has an extraordinary ability to tolerate severe insults of H_2O_2, with rapid recovery of its reduced GSH pool and thence continued

growth. Exploring *in vivo* responses during infection of susceptible (S) and resistant (R gene) host plants reveals that pathogen penetration and proliferation is hugely restricted in the R plants, but surprisingly, there is no change in the roGFP ratio *in planta*. Thus the sparse infection hyphae within R plants maintain a highly reduced cytoplasm at all times. This questions whether production of ROS by the host is the primary mechanism responsible for restricting pathogen growth in resistant plants.

197. Dimorphism and virulence in pathogenic zygomycetes. Soo Chan Lee, Alicia Li, Joseph Heitman. Molec Gen & Microbiol, Duke Med Ctr, Durham, NC.

Fungal dimorphism evolved in multiple fungal lineages. Many pathogenic fungi are dimorphic, for example, switching between yeast and filamentous states. This switch alters host-pathogen interactions and is critical for pathogenicity. However, in pathogenic zygomycetes, whether dimorphism contributes to pathogenesis is a central unanswered question. The pathogenic zygomycete Mucor circinelloides exhibits multi-budded yeast growth under anaerobic/high CO_2 growth conditions, which Louis Pasteur discovered (*Etudes sur la Biere*. 1876). Interestingly, we found that in the presence of the calcineurin inhibitor FK506, Mucor exhibits multi-budded yeast growth. We discovered that Mucor encodes three calcineurin catalytic A subunits (CnaA, CnaB, and CnaC) and one calcineurin regulatory B subunit (CnbR). Disruption of the *cnbR* gene results in mutants locked in yeast phase growth. These results reveal that the calcineurin pathway governs the dimorphic transition from yeast to hyphae. In virulence tests, we found that the *cnbR* yeast-locked mutants are less virulent than wild-type in a heterologous host system, providing evidence that hyphae are a more virulent form of this fungus. Protein kinase A activity was elevated during yeast growth under anaerobic conditions, in the presence of FK506, or in the yeast-locked *cnbR* mutants, indicating a novel connection between PKA and calcineurin. The *cnaA* mutants are hypersensitive to calcineurin inhibitors and display a hyphal polarity defect. The mutants produce spores that are larger than wild-type. Notably, we found spore size is linked to virulence in previous studies (Li et al. *PLoS Pathogens*. 2011). Interestingly, the *cnaA* mutants were found to be more virulent than wild-type. We also observed that the *cnaA* mutants germinate earlier inside macrophages, providing a possible explanation for the greater virulence of the mutants. Another pathogenic zygomycete, *Rhizopus delemar* has three *cna* genes. Phylogenetic analysis revealed that the triplicated *cna* genes might result from a whole genome and/or segmental gene duplications. Our results demonstrate that the calcineurin pathway orchestrates the dimorphic transition, spore size dimorphism, virulence, and hyphal polarity in *Mucor*, and the calcineurin pathway elements have been adapted in zygomycetes via variation in their evolutionary trajectory.

198. Genetic analysis of the components of the *ime-2* mediated signaling events during nonself recognition and programmed cell death (PCD) in *Neurospora crassa*. Joanna A. Bueche[1], Elizabeth A. Hutchison1[1,2], N. Louise Glass[1]. 1) Plant and Microbial Biology, UC Berkeley, Berekeley, CA, 94720; 2) Cornell University Microbiology Department, Ithaca, NY 14853.

Recently, we revealed genetic and functional differences in meiotic initiation machinery between *Neurospora crassa* and *Saccharomyces cerevisiae*. While N. *crassa* is missing some meiotic genes identified in yeast, it has three homologs of the middle meiotic transcriptional regulator, Ndt80. None of the NDT80 homologs are required for meiosis in N. *crassa*. One of the NDT80 homologs, *vib-1* is essential for heterokaryon incompatibility (HI) in N. *crassa*, a nonself recognition mechanism in filamentous fungi. Mutations in *vib-1* suppress cell death caused by HI as well as secretion of the extracellular proteases during the nitrogen starvation. Furthermore, deletion of a IME2 (a kinase involved in initiation of meiosis in S. cerevisiae) homolog in N. *crassa*, *ime-2*, does not affect sexual development, results in a significant elevation of secreted proteases in response to nitrogen starvation. Morever, a D*vib-1* D*ime-2* mutant restored wild-type levels of cell death during the HI and normal production of extracellular proteases; a deletion of *ime-2* suppressed these *vib-1* phenotypes. Based on the evidence, we hypothesize that IME-2 negatively regulates a cell death pathway that functions in parallel to the VIB-1 HI pathway and a protease secretion pathway positively regulated by VIB-1. We used a slightly modified yeast consensus sequence for Ime2 phosphorylation to scan (Scansite) the entire N. crassa genome for possible targets and obtained a list of 30 candidates including VIB-1. All targets were assessed for secretion of the extracellular proteases in absence of nitrogen. Strains containing deletions of 13 of the 30 genes identified in the screen were significantly affected in protease secretion. Mutations in these candidate genes will be tested for the ability to alleviate cell death and Heterokaryon Inocpatibilty (HI) in the presence and absence of *ime-2* and *vib-1* hence assessing their role in the parallel HI/PCD pathway redundant with VIB-1.

199. PRO45 is a component of the conserved STRIPAK complex in *Sordaria macrospora*. Steffen Nordzieke[1], Benjamin Fränzel[2], Sandra Bloemendal[1], Dirk Wolters[2], Ines Teichert[1], Ulrich Kück[1]. 1) General and Molecular Botany, Ruhr-University Bochum; 2) Analytical Chemistry, Ruhr-University Bochum, Universitätsstr. 150, 44801 Bochum, Germany.

The complex formation of three-dimensional fruiting bodies in *Sordaria macrospora* is mediated by an interaction between developmental proteins and conserved signaling cascades and thus an excellent experimental system for developmental biology.

We recently have characterized a STRIPAK complex in *Sordaria macrospora* that is involved in the regulation of fruiting body development. This complex contains striatin (PRO11), a striatin-interacting protein (PRO22), the scaffolding subunit of protein phosphatase 2A (SmPP2AA) and a phocein homologue (SmMOB3) [1, 2].

Here we describe PRO45, a novel subunit of the STRIPAK complex in filamentous fungi which is a homolog of the human sarcolemmal membrane associated protein (SLMAP). We also present the functional characterization of PRO45: Strains lacking the gene for PRO45 show sterility together with a severe defect in hyphal fusion and vegetative growth rate. The primary structure of PRO45 contains a forkhead-associated (FHA) as well as a transmembrane domain. Complementation studies showed that a lack of the FHA domain is responsible for the described defects, whereas a missing transmembrane domain does not affect development.

Tandem affinity purification (TAP) followed by mass spectrometry and coimmunoprecipitation (Co-IP) showed subunits of the STRIPAK-complex as interaction partners, confirming the homology of human and fungal STRIPAK. Further microscopic studies provide evidence for a localization of PRO45 in the ER as well as in the nuclear envelope. Integrating these observations, we propose that PRO45 has a function in the physical and signaling connection of STRIPAK-complex and nucleus.

[1] Pöggeler S, Kück U 2004. Eukaryot. Cell 3: 232-240
[2] Bloemendal S, Lord KM, Rech C, Hoff B, Engh I, Read ND, Kück U. 2010. Eukaryot. Cell 9: 1856-1866
[3] Bloemendal S, Bernhards Y, Bartho K, Dettmann A, Voigt O, Teichert I, Seiler S, Wolters DA, Pöggeler S, Kück U. 2012. Mol. Microbiol. 84: 310-323.

200. Molecular Determinants of Sporulation in *Ashbya gossypii*. Jurgen W. Wendland, Lisa Wasserstroem, Klaus Lengeler, Andrea Walther. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Kopenhagen V, Denmark.

Previously we have analysed the pheromone response MAPK signal transduction cascade in *A. gossypii*. The major findings were (i) deletion of both pheromone receptor genes *STE2* and *STE3* did not inhibit sporulation whereas (ii) deletion of the transcription factor *STE12* resulted in hypersporulation (Wendland *et al*. 2011). Here we present our analysis of key *A. gossypii* homologs of *Saccharomyces cerevisiae* sporulation specific genes. We show that

mutants in *IME1, IME2, KAR4*, and *NDT80* are blocked in sporulation. Mutants in *IME4, KAR4*, and *UME6* also confer a vegetative growth defect. *IME4* expression was found during vegetative growth while *IME2* was not detected under these conditions. We performed transcriptional profiling of non-sporulating strains and determined a core set of about 50 down-regulated sporulation specific genes in these mutants. Interestingly, this set of down-regulated genes is upregulated in the *A. gossypii ste12* mutant providing regulatory evidence of the hypersporulation phenotype of this mutant. Other genes identified in the RNAseq data indicated that during development of sporangia metabolic genes for nutrient uptake are active. Therefore we performed Return-To-Growth assays with mutants inhibited in the sporulation pathway. These strains were kept under conditions in which the wild type initiates sporulation. This lead to induction of sporangium formation, a stage at which these strains remained. Supply of new nutrients resulted in hyphal outgrowth in all mutants indicating that after initiation of the sporulation program *A. gossypii* can reverted to vegetative growth at different stages. In addition we identified differential regulation of two endoglucanases encoded by *ENG1* and *ENG2*. While *ENG1* was not differentially regulated, *ENG2* was down-regulated in e.g. ime1 but strongly up-regulated in *ste12*. Deletion analysis of *ENG2* showed that Eng2 is required for hyphal fragmentation into individual sporangia. We can thus provide a detailed overview of the genetic regulation of sporulation in *A. gossypii*. A comparison with *S. cerevisiae* highlights the role of *KAR4* in sporulation upstream of *IME1*. Finally, our study provides further evidence that the pheromone signaling response MAPK-cascade in *A. gossypii* has a regulatory control function over sporulation alongside regulation of sporulation by nutritional cues.

201. VELVET is regulated by ENV1 and impacts development of *Trichoderma reesei*. Hoda Bazafkan[1], Doris Tisch[2], Monika Schmoll[1]. 1) Health & Environment - Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna.

In *Trichoderma reesei* (teleomorph *Hypocrea jecorina*), light is a crucial environmental factor for initiation of sexual development and considerably influences expression of glycoside hydrolases. In both processes, the light regulatory protein ENV1 plays a key role. Transcriptome analysis revealed that *vel1* (encoding the VELVET orthologue) transcription is regulated by the carbon source in the medium. Moreover, ENV1 negatively regulates *vel1* in light. Genes coregulated with vel1 are enriched in functions of amino acid metabolism as well as carbon metabolism and include three non ribosomal peptide synthases (NRPS). This regulatory pattern supports a connection of *vel1* with primary and secondary metabolism also in *T. reesei*. VELVET is known to be a regulator of sexual and asexual development in fungi. Also for *T. reesei* a function in development was likely, as several genes involved in sexual development are coregulated with *vel1*. Investigation of strains lacking the *T. reesei* orthologue *vel1* under various nutritional, light- and temperature conditions showed that VEL1 is essential for conidiation and growth of aerial hyphae. Moreover, in crossing assays, sexual development with strains lacking *vel1* was delayed and in contrast to the wild-type never occurred in constant darkness. When *vel1* was missing in both mating partners, no fruiting bodies were formed. Although male fertility was intact, female fertility was found to be dependent on the presence of *vel1*. Strains lacking the light regulator gene *env1* are able to undergo sexual development with wild-type strains, but in crosses of two strains lacking *env1*, no fruiting bodies are formed in light. This defect is assumed to be caused by a deregulation of the pheromone system in these strains. Interestingly, also strains lacking *vel1* are unable to mate with *env1* deletion mutants in light. Together with the regulatory connection between these genes, these findings support a function of *vel1* in the same pathway as *env1*. We conclude that VEL1 in *T. reesei* regulates sexual and asexual development and is connected to the light response pathway via ENV1.

202. Sexual reproduction and mating type function in the penicillin producing fungus *Penicillium chrysogenum*. Julia Böhm[1], Birgit Hoff[1], Simon Wolfers[1], Céline O'Gorman[2], Paul Dyer[2], Stefanie Pöggeler[3], Ulrich Kück[1]. 1) Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Deutschland; 2) School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K; 3) Abteilung Genetik eukaryotischer Mikroorganismen, Institut für Mikrobiologie und Genetik, Georg-August Universität Göttingen, 37077 Göttingen, Deutschland.

Penicillium chrysogenum is a filamentous fungus of major medical and historical importance, being the original and present day industrial source of the antibiotic penicillin with a world market value of about 600 million € per year. The species has been considered asexual for over 100 years and despite concerted efforts it has not been possible to induce sexual reproduction. However, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Utilising knowledge of mating-type (*MAT*) gene organization we now describe conditions under which a sexual cycle can be induced leading to the production of cleistothecia and meiotic ascospores, which were similar to those described recently for *Eupenicillium crustaceum* [2]. Evidence of recombination was obtained using both molecular and phenotypic markers. The newly identified heterothallic sexual cycle was used for strain development purposes, generating offspring with novel combinations of traits relevant to penicillin production.

Furthermore, the *MAT1-1-1* mating-type gene, known primarily for a role in governing sexual identity, was also found to control transcription of a wide range of genes including those regulating penicillin production, hyphal morphology and conidial formation, all traits of biotechnological relevance. For functional characterization *MAT1-1-1* knockout and overexpression strains were generated and analyzed. These discoveries of a sexual cycle and *MAT* gene function are likely to be of broad relevance for manipulation of other asexual fungi of economic importance.

[1] Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, Fleming`s *Penicillium* strain discloses the secret of its sex. Eukaryot Cell 7: 465-470

[2] Pöggeler S, O'Gorman CM, Hoff B, Kück U (2011) Molecular organization of the mating-type loci in the homothallic ascomycete *Eupenicillium crustaceum*. Fungal Biol. 115: 615-624.

203. Exponentiate complexity: non-mating GPCRs in the basidiomycete *Schizophyllum commune*. Daniela Freihorst, Susann Erdmann, Erika Kothe. Institute for Microbiology, Dept. Microbial Communication, Friedrich Schiller University, Jena, Germany.

The filamentous fungus *S. commune* is a model organism for sexual development of basidiomycetes. Numerous studies revealed the importance of two gene loci, *A* and *B*, responsible for tetrapolar mating and sexual development. Both occur in multiallelic subloci leading to a large number of different specificities in nature (9 to 32 depending on locus), which then control compatibility or abortion of mating. While *A* codes for homeodomain transcription factors, *B* codes for a pheromone/receptor system. The *B*-receptors (Ste3-like, seven transmembrane domains, G-protein coupled) recognize pheromones of non-self specificity and induce signal transduction pathways and specific gene regulation. After sequencing of strain H4-8 four new Ste3-like GPCRs, homologous to the known *B*-specific ones, were found. Three of the four *B*-receptor like genes (*brls*) are located close to the *B*-locus. Their function is unknown, because a *B*-locus defective strain without any interactions seen in *B*-dependent development still contains those four GPCRs, which obviously do not respond to any wild type pheromone. However, our results indicate their importance since sequence identity - analyzed by PCR and sequencing - between unrelated strains was found arguing for conservation of these genes. Gene expression was first observed by Reverse Transcriptase PCR as well as Microarray analyses, which disproved the theory that *brls* are pseudo genes. Expression was then investigated by quantitative Real Time PCR during mating interaction and in monokaryotic strains, which showed comparable results only between gene *brl4* and the true mating receptor *bar2*. Also RNA

Seq data did not substantiate a mating dependent expression for those genes. To get more insights into the function an over expression of the gene *brl2* under control of *tef1*-promoter was performed. Phenotypes of independent mutants showed no hints for a faster mating, changes in clamp formation or nuclear distribution tested by mating experiments. Only an asymmetric distribution of fruiting bodies was visible, which seems to originate from the different protein background respectively epigenetics of the two partners just before mating interaction and dikaryotization. Tagging of the receptors for visualization is planned, which will lead to more knowledge about localization and putative interacting proteins.

204. Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete *Sordaria macrospora*. Britta Herzog, Yasmine Bernhards, Berit Habing, Eva Reschka, Sabine Riedel, Stefanie Pöggeler. Institute of Microbiology and Genetics, Department of Genetics of Eurkaryotic Microorganisms, Georg-August-University Göttingen, Germany.

Using *Sordaria macrospora* as model organism we investigate the complex process of fruiting-body development and involved proteins in this filamentous ascomycete. This differentiation process is regulated by more than 100 developmental genes. Recently, we have shown that a homologue of the human STRIPAK (striatin-interacting phosphatase and kinase) complex engages a crucial role in sexual development in fungi. The *S. macrospora* striatin homologue PRO11 and its interaction partner SmMOB3 are key components of this complex (Bloemendal *et al*., 2012). PRO11 contains a conserved WD40 repeat domain and is supposed to function as scaffolding protein linking signaling and eukaryotic endocytosis (Pöggeler and Kück, 2004). SmMOB3 (phocein) is a member of the MOB family (Bernhards and Pöggeler, 2011). Beside their important role in multicellular development and hyphal fusion both proteins seem to be involved in vesicular trafficking and endocytosis.

By means of yeast two-hybrid screens and GFP-Trap analysis we identified several new interaction partners of PRO11 and SmMOB3. Similar to PRO11 and SmMOB3, a multitude of them are predicted to be involved in vesicular trafficking and are localized to the ER or to the Golgi. Here, we show the results of a detailed analysis of the new STRIPAK complex interaction partners. Initially, we isolated the cDNA of the genes and confirmed the interaction by yeast two-hybrid. For further characterization and to get knowledge about their cellular functions we created knock-out strains and analyzed their morphological phenotypes. For localization and expression studies we constructed EGFP-tagged fusion proteins and expressed them in *S. macrospora*.

Bernhards and Pöggeler, 2011; Curr Genet 57 (2): 133-49.
Bloemendal *et al*., 2012; Mol Microbiol 84 (2): 310-23.
Pöggeler and Kück, 2004; Eukaryot Cell 3 (1): 232-40.

205. Hypocrea jecorina meiosis generates segmentally aneuploid progeny to enhance production of xylan-degrading hemicellulases. T.-F Wang, C.L Chen, P. W.-C. Hsu, W.-C. Li, S.-Y. Tung, C.-L. Wang, H.-C. Kuo. Institute of Molecular Biology, Academia Sinica, Taipei, TaiwanI.

Hypocrea jecorina is the sexual form of Trichoderma reesei, an industrially important cellulolytic filamentous fungus. We report that H. jecorina meiosis utilizes a novel Ku70-dependent duplication mechanism to generate segmentally aneuploid progenies, thus increasing the diversity of genotypes and ensuring more efficient xylan degradation. H. jecorina sexual reproduction yields hexadecad asci with 16 linearly arranged ascospores. Our results indicate that these ascospores are generated via two rounds of postmeiotic mitosis following the two meiotic divisions. Remarkably, the hexadecad asci frequently (>90%) contain four or eight inviable ascospores with an equal number of viable segmentally aneuploid ascospores. Array-based comparative genomic hybridization revealed that all the viable segmental aneuploid progenies have a large chromosomal duplication (~0.5Mbp). Deletion of the nonhomologous end-joining gene ku70 restores canonical meiosis and 16 viable euploid ascospores. Segmental duplication contains genes involved in xylan degradation and enhances expression of several carbohydrate-active enzymes, particularly cell wall degrading hemicellulases.

206. Deletion of MAT 1-2-1 gene results in mating type switching in *Ceratocystis fimbriata*. P. Markus Wilken[1], Emma T. Steenkamp[2], Mike J. Wingfied[1], Z. Wilhelm de Beer[2], Brenda D. Wingfield[1]. 1) Dept Genetics, University of Pretoria, Pretoria, Gauteng, South Africa; 2) Dept Microbiology and Plant Pathology, University of Pretoria, Pretoria, Gauteng, South Africa.

Sequencing of the *Ceratocystis fimbriata* genome has made it possible to consider the long standing question as to how uni-directional mating type switching functions in this fungal pathogen and its relatives. Uni-directional mating type switching was first observed in the homothallic ascomycete *C. fimbriata* in the 1960's. Two forms of progeny arise after meiosis, some self-fertile and thus not requiring an opposite mating partner to complete the sexual cycle. Other isolates are self-sterile and unable to reproduce sexually. This loss of self-fertility has been shown to be associated with the loss of a fragment of the mating specific gene, *MAT1-2-1*, in self-sterile strains. The aim of this study was interrogate the full genome sequence of *C. fimbriata* to determine whether the full *MAT1-2-1* gene is deleted and whether other MAT genes are affected during mating type switching. We were able to determine that *C. fimbriata* has both the *MAT1-2-1* gene and the MAT1-1 genes (*MAT1-1-1* and *MAT1-1-2*). The self-sterile isolates had only lost the *MAT1-2-1* gene and one copy of a 230 base pair perfect repeat which flanks this gene in the self fertile isolates. The loss of the entire *MAT1-2-1* gene explains the loss of fertility and the repeats are suggestive of the involvement of recombination during the deletion event. This study illustrates a unique mating strategy in the fungi, not previously understood at the molecular level. The newly gained knowledge will also make it possible to consider the mechanisms underpinning uni-directional switching in other species of *Ceratocystis*.

207. Mannitol is essential for the development of stress resistant ascospores in *Neosartorya fischeri*. Timon T. Wyatt[1], M.R. van Leeuwen[1], H.A.B. Wösten[2], J. Dijksterhuis[1]. 1) Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; 2) Microbiology, Utrecht Univeristy, Utrecht, the Netherlands.

The sugar alcohol mannitol is one of the main compatible solutes in *Neosartorya fischeri* and accumulates especially in conidia and ascospores. In fungi, mannitol has been implicated in a wide variety of functions including carbon storage, maintaining reduction potential, water absorption, heat stress protection, protection against oxidative stress, and tolerance against osmotic stress. Biosynthesis of mannitol in ascomycota mainly depends on mannitol 1-phosphate dehydrogenase (MPD). In our study a functional analysis was performed of the MPD encoding gene *mpdA* of *N. fischeri*. The fluorescence proteins GFP and dTomato were put under control of the *mpdA* promoter. Expression of *mpdA* was observed in aerial hyphae and conidiophores, but was especially high in ascomata and ascospores. Disruption of *mpdA* reduced mannitol as much as 85% of the wild type and increased trehalose levels to more than 400%. Decreased mannitol accumulation had no obvious effect on mycelium growth when exposed to temperature and oxidative stress, while an increased stress sensitivity of conidia against heat and oxidative stress was observed. The most distinct phenotype of *mpdA* disruption was the complete absence of ascospores. Formation of fruiting bodies (ascomata) and asci was not affected but the developmental defect was shown to occur after meiosis. Similar results were obtained by adding the MPD inhibitor nitrophenide to the wild-type strain. Our result suggest a role of mannitol as carbon storage molecule during sexual development, but also its role as scavenger of hydroxyl radicals can be of importance for the formation of sexual spores. Mannitol might regulate the Reactive Oxygen Species (ROS) levels induced by Nox (NADPH oxidases) family enzymes during sexual development. Taken together,

these results show a novel function for mannitol in fungal growth and sexual development.

208. A small lipopeptide pheromone with limited proline substitutions can still be active. Thomas J. Fowler, Stephanie L. Link. Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL.

Mating in many fungi involves communication with lipopeptide pheromones. These signaling molecules activate G protein-coupled receptors located on the surface of a compatible mating partner and initiate a mating response. Some of the mushroom fungi code for scores of different lipopeptide pheromones among the mating types. Despite their small predicted size of approximately eleven amino acids, these pheromones have very specific pheromone receptor targets for mate discrimination. In past heterologous expression and mating studies in *Saccharomyces cerevisiae*, we have made random amino acid substitutions in one pheromone, Bbp2(4), from the mushroom fungus *Schizophyllum commune*, and site-directed mutations in a closely related pheromone, Bbp2(7). These studies indicated that the peptide portion of the pheromones can be more variable than expected. Within a random mutagenesis study of Bbp2(4), it was noted that the imino acid proline could be substituted for several of the natural residues and an active mutant pheromone was still produced. In this study, the heterologous mating assay was employed to test the extent that proline residues might be substituted into a pheromone before activity was no longer detected. Mature Bbp2(4) is predicted to be eleven amino acids with a farnesyl tail (DSPDGYFGGYC-farnesyl). Single substitutions of proline at several non-natural positions did not stop production of active pheromone, but substitutions with proline at several previously identified critical amino acid positions led to negative results in the mating assays. Among the substitutions that do not disrupt all activity are DSP*P*GYFGGYC-farnesyl and DSPDGYFG*P*YC-farnesyl. The three-dimensional conformations of proline-substituted peptides in solution were predicted with PEP-FOLD and viewed with JMOL. The conformational differences of small pheromones tolerated by one receptor are surprising. Substitution of two or more prolines at adjacent non-natural positions in a single pheromone does inhibit production of an active pheromone in the heterologous mating assay. At present, it cannot be determined if multiple proline substitutions inhibit pheromone processing, pheromone transport, or interaction with the receptor.

209. Function of Ras proteins in fungal morphogenesis of *Schizophyllum commune*. E.-M. Jung, N. Knabe, E. Kothe. Department of Microbiology, Friedrich Schiller University, Jena, Germany.

The white rot basidiomycete *Schizophyllum commune* has been used as a model organism to study mating and sexual development as well as analysis of cell development. Subsequent to nutrient and pheromone recognition, intracellular signal transduction was regulated by different pathways and MAPK signalling cascades. The *S. commune* genome encodes more than 30 putative signal transduction proteins of the Ras superfamily containing the Ras, Rho, Rab, Ran and Arf subfamilies. Phylogenetic investigation of Ras proteins from various basidiomycetes show that they cluster in two main groups. High sequence similarities between these proteins in basidiomycetes suggesting an ancient duplication event. To investigate the function of the small G-proteins Ras1 and Ras2 mutants with constitutively active *ras1* alleles as well as a DRasGap1 mutant were analyzed. They show phenotypes with disorientated growth pattern, reduced growth rates and hyperbranching effects. The fungal cytoskeleton, composed of actin and microtubules has been investigated by immunofluorescence microscopy to reveal whether Ras signaling influences the formation of cytoskeleton. The second Ras protein, Ras2, was detected by genome analysis. Its function is analysed in current studies.

210. The developmental PRO40/SOFT protein participates in signaling via the MIK1/MEK1/MAK1 module in *Sordaria macrospora*. Ines Teichert[1], Eva Steffens[1], Nicole Schnab[1], Benjamin Fränzel[2], Christoph Krisp[2], Dirk A. Wolters[2], Ulrich Kück[1]. 1) General & Molecular Botany, Ruhr University Bochum, Bochum, Germany; 2) Analytical Chemistry, Ruhr University Bochum, Bochum, Germany.

Filamentous fungi are able to differentiate multicellular structures like conidiophores and fruiting bodies. Using the homothallic ascomycete *Sordaria macrospora* as a model system, we have identified a number of developmental proteins essential for perithecium formation. One is PRO40 [1], the homolog of *Neurospora crassa* SOFT, and this protein was employed for protein-protein interaction studies to gain insights into its molecular function. Data from yeast two hybrid experiments with PRO40 as bait show an interaction of PRO40 with the MAP kinase kinase (MAPKK) MEK1. MEK1 is a member of the cell wall integrity (CWI) pathway, one of three MAP kinase modules present in *S. macrospora*. The *S. macrospora* CWI pathway consists of MAP kinase kinase kinase (MAPKKK) MIK1, MAPKK MEK1 and MAP kinase (MAPK) MAK1, with additional upstream components, protein kinase C (PKC1) and RHO GTPase RHO1. Data from tandem affinity purification - MS experiments with PRO40 and MEK1 as bait indicate that PRO40 forms a complex with components of the CWI pathway. Analysis of single and double knockout mutants shows that PRO40, MIK1, MEK1 and MAK1 are involved in the transition from protoperithecia to perithecia, hyphal fusion, vegetative growth, and cell wall stress response. Differential phosphorylation of MAPKs in a pro40 knockout strain was detected by Western analysis. We propose that PRO40 modulates signaling through the CWI module in a development-dependent manner. Further interaction studies and complementation analyses with PRO40 derivatives provide mechanistic insight into the function of PRO40 domains during fungal development. [1] Engh et al. (2007) Eukaryot Cell 6:831-843.

211. Map-based identification of the *mad* photosensing genes of *Phycomyces blakesleeanus*. Silvia Polaino Orts[1], Suman Chaudhary[1], Viplendra Shakya[1], Alejandro Miralles-Durán[2], Luis Corrochano[2], Alexander Idnurm[1]. 1) Cell Biology & Biophysics, University of Missouri-KC, Kansas City, MO; 2) Departamento de Genética, Universidad de Sevilla, Spain.

Phycomyces blakesleeanus is a filamentous fungus, a member of the subphylum Mucoromycotina. The main reason for the presence of *Phycomyces* in laboratories is its sensitivity to light. The fruiting bodies phototropism of *Phycomyces* has served as a model of response to blue light in fungi. In 1967, in the laboratory of Nobel laureate Max Delbrück, the first sensory mutants were isolated. Analysis on these strains has enabled a proposed sensory transduction pathway that describes the flow of information from the sensors to the effectors. There are ten mutants, called *mad* mutants, divided into two classes: those of type 1 are *madA*, *madB*, *madC* and *madI*, which are altered only in photoresponses but not in others tropisms of the sporangiophore. The mutants in the *madA* and *madB* genes are altered in all photoresponses (phototropism, photomorphogenesis, photocarotenogenesis and photomecism). These two *mad* genes are the only ones that have been identified and their corresponding proteins interact to form the Mad complex, the main photoreceptor complex of *Phycomyces*. The mutants altered in the *madC* gene are only affected in the phototropism. The remaining *mad* mutants are called type 2 and are altered in the phototropism and other responses of the sporangiophore, like gravitropism and avoidance. *Phycomyces* cannot be stability transformed with DNA. To identify the eight unknown *mad* mutants, a positional cloning approach was taken coupled to Illumina sequence information. A genetic map was constructed between two wild type parents, and then *mad* mutants crossed to one of these parents. Through mapping, we have identified candidates for the *madC*, *madD*, *madJ*, *madF* and *madI* genes, with greatest follow up characterization in *madC*. The *madC* gene encodes a Ras GTPase-activating protein, implicating Ras in the light signal transduction pathway in fungi.

212. The C_2H_2 transcription factor HgrA promotes hyphal growth in the dimorphic pathogen *Penicillium marneffei*. Hayley E. Bugeja, Michael J. Hynes, Alex Andrianopoulos. Department of Genetics, University of Melbourne, Parkville, VIC, Australia.

Penicillium marneffei (recently renamed *Talaromyces marneffei*) is well placed as a model experimental system for investigating fungal growth processes and their contribution to pathogenicity. An opportunistic pathogen of humans, *P. marneffei* is a dimorphic fungus that displays multicellular hyphal growth and asexual development (conidiation) in the environment at 25°C and unicellular fission yeast growth in macrophages at 37°C. We have characterised the transcription factor *hgrA* (hyphal growth regulator), which contains a C_2H_2 DNA binding domain closely related to that of the stress-response regulators Msn2/4 of *Saccharomyces cerevisiae*. HgrA is not required for controlling yeast growth in response to the host environment, nor does it appear to have a key role in response to stress agents, but is both necessary and sufficient to drive the hyphal growth program. *hgrA* expression is specific to hyphal growth and its deletion affects multiple aspects of hyphal morphogenesis and the dimorphic transition from yeast cells to hyphae. Loss of HgrA also causes cell wall defects, reduced expression of cell wall biosynthetic enzymes and increased sensitivity to cell wall, oxidative, but not osmotic stress agents. As well as causing apical hyperbranching during hyphal growth, overexpression of *hgrA* prevents conidiation and yeast growth, even in the presence of inductive cues. HgrA is a strong inducer of hyphal growth and its activity must be appropriately regulated to allow alternative developmental programs to occur in this dimorphic pathogen.

213. Involvement of a specific ubiquitin ligase in the assembly of the dynein motor. Ryan Elsenpeter, Robert Schnittker, Michael Plamann. Sch Biological Sci, Univ Missouri, Kansas City, Kansas City, MO.

Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargoes. The dynein heavy chain (DHC) is >4000 residues in length, with the last two-thirds of the heavy chain forming the motor head. Six domains within the dynein motor exhibit varying degrees of homology to the AAA+ superfamily of ATPases. These domains form a ring-like structure from which a microtubule-binding domain protrudes. Using a genetic assay, we have isolated over 30 DHC mutants of Neurospora that produce full-length proteins that are defective in function. To explore the mechanism by which mutations in the C-terminal region of the DHC affect function, we have identified both intragenic and extragenic suppressors. Interestingly, analysis of the extragenic suppressors revealed that loss of function for a putative E3 ubiquitin ligase restored dynein function in a select set of C-terminal DHC mutants. Our results suggest that these C-terminal DHC mutations block assembly of the dynein motor and loss of activity of a specific E3 ubiquitin ligase restores dynein assembly.

214. Identification and characterization of new alleles required for microtubule-based transport of nuclei, endosomes, and peroxisomes. K. Tan, A. J. Roberts, M. Chonofsky, M. J. Egan, S. L. Reck-Peterson. Dept Cell Biology, Harvard Medical School, Boston, MA.

Eukaryotic cells use the microtubule-based molecular motors dynein and kinesin to transport a wide variety of cargos. Cytoplasmic dynein is responsible for minus-end-directed microtubule transport (from the cell periphery towards the nucleus), while kinesins-1, -2 and -3 move cytoplasmic cargo in the opposite direction. While much is known about how these motors work *in vitro*, many questions regarding the mechanism and regulation of microtubule-based cargo transport in cells remain. To identify novel alleles and genes required for microtubule-based transport, we have performed a genetic screen in the filamentous fungus, *Aspergillus nidulans*. We fluorescently-labeled three different organelle populations that are known to be cargo of dynein and kinesin in *Aspergillus*: nuclei, endosomes, and peroxisomes. After mutagenesis we used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Here, we report the identification and characterization of new alleles of kinesin, dynein and the dynein regulatory factors, Lis1 and Arp1 (a component of the dynactin complex). *In vivo* analysis of two new dynein alleles revealed that mutations in two of dynein's nucleotide binding sites (termed AAA1 and AAA3), led to the accumulation of endosomes and peroxisomes at the hyphal tip, with more subtle defects on nuclear distribution compared to dynein null alleles. *In vitro* studies of the AAA3 motor mutation showed dramatic reduction in velocity and prolonged binding to the microtubules in single molecule motility assays.

215. Pheromone-induced G2 cell cycle arrest in Ustilago maydis requires inhibitory phosphorylation of Cdk1. Sónia M. Castanheira, José Perez-Martín. Centro Nacional de Biotecnología. CSIC. Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain.

Ustilago maydis is a dimorphic basidiomycete that infects maize. In this fungus virulence and sexual development are intricately interconnected. Induction of pathogenicity program requires that two haploid compatible cells fuse and form an infective filament after pheromone signaling. The pheromone signal is transmitted by a well-known MAPK cascade. Interestingly, Saccharomyces cerevisiae and Ustilago maydis use a similar MAPK cascade to respond to sexual pheromone and in both cases a morphogenetic response is provided (shmoo and conjugative hypha, respectively). However, while S. cerevisiae arrests its cell cycle in G1 in response to pheromone, U. maydis does this by arresting at G2. The mechanisms and physiological reasons involved in the distinct cell cycle response to pheromone in U. maydis are largely unknown. In this communication we will introduce our attempts to characterize the molecular mechanisms behind pheromone-induced cell cycle arrest in U. maydis .Our results have indicated that inhibitory phosphorylation of Cdk1 is part of the mechanism of the pheromone-induced G2 cell cycle arrest. This inhibitory phosphorylation depends on the essential kinase Wee1. We analyzed the transcriptional pattern of cell cycle related genes in response to overactivation of pheromone pathway (using a constitutively activated allele of fuz7, the MAPKK of the cascade) and found that two main G2/M regulators -Hsl1, a kinase involved in downregulation of Wee1 and Clb2, the mitotic cyclin- were downregulated at transcriptional level. Using chimeric promoter fusions we found that transcriptional downregulation was not as important for pheromone-induced cell cycle arrest as expected and we are analyzing other possible regulatory options such as stability or subcellular localization of these regulators.

216. Microtubule-dependent mRNA transport and mitochondrial protein import in Ustilago maydis. T. Langner[1], T. Pohlmann[1], C. Haar[1], J. Koepke[2], V. Goehre[1], M. Feldbruegge[1]. 1) Institute for Microbiology, Heinrich-Heine University, Duesseldorf, Northrhine-Westfalia, Germany; 2) MARA, Philipps-University, Marburg, Hesse, Germany.

Transport, subcellular localization, and local translation of mRNAs constitute a very important mechanism to ensure correct targeting of proteins to distinct subcellular domains. Although mRNA transport is well studied in various organisms, its function in regulating specific cellular processes like mitochondrial protein import is still ambiguous. We use the corn pathogen Ustilago maydis as a model system to study microtubule-dependent mRNA transport during formation of infectious filaments. The key RNA-binding protein Rrm4 is an integral part of this long-distance transport machinery. Combining proteomics, in vivo UV cross-linking, and biochemical approaches, we uncovered that Rrm4 plays a crucial role in active transport of mRNAs encoding mitochondrial proteins. In Rrm4 loss-of-function mutants, mitochondrial proteins are altered in expression and localization, which correlates with impaired production of reactive oxygen species (ROS). We propose that microtubule-dependent mRNA transport and local translation are crucial for correct import of mitochondrial proteins. This work is funded by iGRAD-plant graduate school (German research council, DFG/ GRK1525).

217. "The vacuole" of Neurospora crassa may be composed of multiple compartments with different structures and functions. Barry J. Bowman[1], Emma Jean Bowman[1], Robert Schnittker[2], Michael Plamann[2]. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Department of Biology, University of Missouri, Kansas City, KA.

The structure of the "vacuole" in *Neurospora crassa* and other filamentous fungi is highly variable with cell type and position in the hypha. Large spherical vacuoles are typically observed in older hyphal compartments, but approximately 100 microns behind the hyphal tip, vacuolar markers are seen in a dynamic network of thin tubules. At the edge of this network nearest the tip, a few distinct round organelles of relatively uniform size (2-3 microns) have been observed (Bowman *et al.* Eukaryotic Cell 10:654). The function of these round organelles is unknown, although the vacuolar ATPase and a vacuolar calcium transporter are strongly localized there. To help identify organelles we have tagged SNARE proteins and Rab GTPases with GFP and RFP. Several of these tagged proteins (sec-22, rab-7, rab-8) appear in the tubular vacuolar network and in the membrane of the round organelles. A unique aspect of the round organelles is their association with dynein and dynactin (Sivagurunathan *et al.* Cytoskeleton, 69:613). In strains with mutations in the tail domain of the dynein heavy chain the dynein is often seen in clumps. This aggregated dynein appears to be tightly associated with (and possibly inside) the round organelles, but not in the tubular vacuolar network. Further analysis of the location of SNARE and Rab proteins may help to identify the function of the round organelles.

218. Comparisons of two wild type *A* mating type loci and derived self-compatible mutants in the basidiomycete *Coprinopsis cinerea*. Yidong Yu, Monica Navarro-Gonzaléz, Ursula Kües. Molecular Wood Biotechnology + Technical Mycology, University of Goettingen, Goettingen, Germany.

The *A* mating type locus in *Coprinopsis cinerea* controls defined steps in the formation of a dikaryotic mycelium after mating of two compatible monokaryons as well as fruiting body formation on the established dikaryon. Usually, three paralogous pairs of divergently transcribed genes for two distinct types of homeodomain transcription factors (termed HD1 and HD2) are found in the multiple alleles of the *A* locus. For regulation of sexual development, heterodimerization of HD1 and HD2 proteins coming from allelic gene pairs is required. In some *A* loci found in nature, alleles of gene pairs are not complete or one of the two genes have been made inactive. Functional redundancy allows the system still to work as long as an *HD1* gene in one and an *HD2* gene in the other allele of one gene pair are operative (Casselton and Kües 2007). Here, we present the structures of two completely sequenced *A* loci, *A42* (this study) and *A43* (Stajich et al. 2010). Evidences for gene duplications, deletions and inactivations are found. The loci differ in the number of potential gene pairs (five versus three), in genes that have been duplicated in evolution, in genes that have been lost in evolution and in genes that are still present but have been made inactive. Furthermore, self-compatible mutants of the *A* loci are found that due to fusions of an *HD1* and an *HD2* gene can carry out sexual reproduction without mating with another compatible strain. The products of the fusion genes can take over the regulatory functions normally executed by heterodimers of HD1 and HD2 proteins that come from different nuclei. In this study, we present a sequenced fusion gene from a mutant *A43* locus. The 5'-half of an *HD2* gene was fused in frame to a complete *HD1* gene through a linker made up from former promoter sequence. An earlier described fusion protein (Kües et al. 1994) similarly contains the 5'-half of an *HD2* gene that however was fused to the 3'-half of an *HD1* gene. Comparison between the resulting fusion proteins indicates that presence of the HD2 homeodomain and the NLSs (nuclear localization signals) from the HD1 protein are likely essential for the function of the fusion proteins. Other domains required for function in the wild type proteins (such as for heterodimerization) are dispensable for fusion proteins that mediate a self-compatible phenotype.

219. Transformation of an NACHT-NTPase gene *NWD2* suppresses the *pkn1* defect in fruiting body initiation of the *Coprinopsis cinerea* mutant Proto159. Yidong Yu[1], Pierre-Henri Clergeot[2], Gwenäel Ruprich-Robert[3], Markus Aebi[4], Ursula Kües[1]. 1) Molecular Wood Biotechnology + Technical Mycology, University of Goettingen, Goettingen, Germany; 2) Department of Botany, Stockholm University, Stockholm, Sweden; 3) Institute of Genetics and Microbiology, University Paris-Sud, Orsay, France; 4) Institute of Microbiology, ETH Zurich, Zurich, Switzerland.

Homokaryon AmutBmut is a self-compatible strain of the mushroom *Coprinopsis cinerea* which can carry out sexual reproduction without fusing with another compatible strain. Due to its single nucleus, this strain allows easy induction of mutations in fruiting body formation. One such mutant is the strain Proto159, which is defective in the first step of fruiting body initiation (primary hyphal knot formation; *pkn1*). This mutant has been isolated after protoplasting and regeneration of oidia (Granado et al. 1997). It has a reduced growth speed and a reduced rate of oidiation (asexual spore formation) compared to the wild type AmutBmut. In addition, with age, the mycelium of Proto159 produces a dark-brown pigment that diffuses into the medium. This pigmentation is not found in AmutBmut. Proto159 never makes any sclerotium nor initiates formation of any fruiting structure. Complementation tests have been made through transformations with a cosmid bank of the wild type AmutBmut (Bottoli et al. 1999) and the defect has been complemented after transformation with the wild type gene *NWD2*. This gene codes for a NACHT-NTPase (signal transduction protein with a NACHT domain which is found in animal, fungal and bacterial proteins and named after four different types of P-loop NTPases NAIP, CIITA, HET-E and TP1). However, sequencing of this gene in the mutant Proto159 did not reveal any point mutations, deletions or insertions within this gene. One possibility to explain the *pkn1* defect in mutant Proto159 in connection with the transformation data is that insertion of further copies of gene *NWD2* into the genome of mutant Proto159 has a suppressor effect on the defect in the yet unknown gene *pkn1*. This situation is reminiscent to findings in *Schizophyllum commune* where formation of fruiting bodies has been induced in monokaryons upon transformation with the gene *Frt1* (Horton and Raper 1991). Gene *Frt1* encodes another type of P-loop NTPase (Horton and Raper 1995) than *NWD2*. However, the proteins share a novel short motif of amino acid similarity at their C-terminal ends.

220. Dynamics of the actin cytoskeleton in *Phytophthora infestans*. Harold Meijer[1], Chenlei Hua[1], Kiki Kots[1,2], Tijs Ketelaar[2], Francine Govers[1]. 1) Lab Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Lab Cell Biology, Wageningen University, Wageningen, Netherlands.

The actin cytoskeleton is conserved among all eukaryotes and plays essential roles during many cellular processes. It forms an internal framework in cells that is both dynamic and well organised. The plethora of functions ranges from facilitating cytoplasmic streaming, muscle contraction, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. Oomycetes are filamentous organisms that resemble Fungi but are not related to Fungi. The two groups show significant structural, biochemical and genetic differences. One prominent lineage within the class of oomycetes is the genus *Phytophthora*. This genus comprises over 100 species that are all devastating plant pathogens threatening agriculture and natural environments. The potato late blight pathogen *Phytophthora infestans* was responsible for the Irish potato famine and remains a major threat today. Previously the actin organization has been studied in several oomycetes. Next to the common F-actin filaments and cables, cortical F-actin containing patches or plaques have been observed as in Fungi. However, only a static view was obtained. Here, we use an in vivo actin binding moiety labelled to a fluorescent group to investigate the actin cytoskeleton dynamics in hyphae of *P. infestans*. Our results provide the first visualisation of the dynamic reorganization of the actin cytoskeleton in oomycetes. In the future, this line will provide insight in the role of the actin cytoskeleton during infection.

Comparative and Functional Genomics

221. A novel approach for functional analysis of genes in the rice blast fungus. Sook-Young Park[1], Jaehyuk Choi[1], Seongbeom Kim[1], Jongbum Jeon[1], Jaeyoung Choi[1], Seomun Kwon[1], Dayoung Lee[1], Aram Huh[1], Miho Shin[1], Junhyun Jeon[1], Seogchan Kang[2], Yong-Hwan Lee[1]. 1) Dept. of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea; 2) Dept. of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802, USA.

Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in *Magnaporthe oryzae* by constitutive expression under a strong promoter. Employing a binary vector (pGOF), carrying *EF1b* promoter, we generated a total of 4,432 transformants by *Agrobacterium tumafaciens*-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, *MoRPT4* and *MoDBP5*, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformant library would be a valuable resource for functional characterization of the redundant or lethal genes in *M. oryzae* and this system may be applicable in other fungi.

222. Distribution and evolution of transposable elements in the Magnaporthe oryzae/grisea clade. Joelle Amselem[1,2], Ludovic Mallet[1,3], Helene Chiapello[3,4], Cyprien Guerin[3], Marc-Henri Lebrun[2], Didier Tharreau[5], Elisabeth Fournier[6]. 1) INRA, URGI, Versailles, France; 2) INRA, UMR BIOGER, Thiverval-Grignon, France; 3) INRA, UR MIG, Jouy-en-Josas, France; 4) INRA, UR BIA, Castanet-Tolosan, France; 5) CIRAD, UMR BGPI, Montpellier, France; 6) INRA, UMR BGPI, Montpellier, France.

Magnaporthe oryzae is a successful pathogen of crop plants and a major threat for food production. This species gathers pathogens of different Poaceaes, and causes the main fungal disease of rice worldwide and severe epidemics on wheat in South America. The evolutionary genomics of Magnaporthe oryzae project aims at characterizing genomic determinants and evolutionary events involved in the adaptation of fungus to different host plants. Such evolution may rely on variations in Transposable Elements (TEs) and gene content as well as modification of coding and regulatory sequences. Indeed, TEs are essential for shaping genomes and are a source of mutations and genome re-organizations. We performed a comparative analysis of TEs in 9 isolates from the M. oryzae/grisea clade differing in their host specificity using a reference TEs consensus library (Mg7015_Refs_TE) made from M. grisea 70-15 reference genome. We used REPET pipelines (http://urgi.versailles.inra.fr/Tools/REPET) to detect ab initio and classify TEs in *M. grisea* 70-15 according to functional features (LTR, ITR, RT, transposase, etc.). After manual curation on consensus provided by the TEdenovo pipeline, we used the resulting consensus of TE families (Mg7015_Refs_TE) to annotate the 9 genome copies including nested and degenerated ones using TEannot pipeline. We will present results obtained for Mg7015_Refs_TE classification, their annotation, distribution along the genome and preliminary results provided by comparison in *M. oryzae/grisea* species studied regarding correlation with phylogeny and host specificity.

223. Alternative structural annotation of Aspergillus oryzae and Aspergillus nidulans based on RNA-Seq evidence. Gustavo C Cerqueira[1], Brian Haas[1], Marcus Chibucos[2], Martha Arnaud[3], Christopher Sibthorp[4], Mark X Caddick[4], Kazuhiro Iwashita[5], Gavin Sherlock[3], Jennifer Wortman[1]. 1) Broad Institute, Boston, MA; 2) Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA; 3) Department of Genetics, Stanford University Medical School, Stanford, USA; 4) School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom; 5) National Research Institute of Brewing, Hiroshima, Japan.

The correct structural annotation of genes is fundamental to downstream functional genomics approaches. Genes undetected by gene prediction algorithms, incorrect gene boundaries, misplaced or missing exons and wrongly merged genes can jeopardize attempts to produce a comprehensive catalog of an organism's metabolic capabilities. We are currently working toward generating alternative and improved structural annotation of Aspergillus oryzae and Aspergillus nidulans. Our approach consists of assembling partial transcript sequences from RNA-Seq data, aligning transcript assemblies to their respective genomic loci and finally adjusting the gene models according to the new trancript evidence. Novel putative genes were defined based on transcriptionally active regions containing splice junctions and open reading frames. Gene loci having transcripts suggesting alternative splicing variants were reported. The nucleotide composition in the vicinity of splicing sites was re-evaluated in the light of the newly defined exons-introns boundaries. The modified structural annotation was compared to the original structural annotation of these genomes and alternative gene models derived from approaches similar to those presented here. The improved gene models are available through the Aspergillus genome database (http:// http://www.aspergillusgenome.org).

224. Improved Gene Ontology annotation for biofilm formation, filamentous growth and phenotypic switching in *Candida albicans*. Diane O. Inglis, Marek S. Skrzypek, Arnaud B. Martha, Binkley Jonathan, Prachi Shah, Farrell Wymore, Gavin Sherlock. Department of Genetics, Stanford University, Stanford, CA.

The opportunistic fungal pathogen, *Candida albicans*, is a significant medical threat, especially for immunocompromised patients. Experimental research has focused on specific areas of *C. albicans* biology with the goal of understanding the multiple factors that contribute to its pathogenic potential. Some of these factors include cell adhesion, invasive or filamentous growth and the formation of drug resistant biofilms. The *Candida* Genome Database (CGD, http://www.candidagenome.org/) is an internet-based resource that provides centralized access to genomic sequence data and manually curated functional information about genes and proteins of the fungal pathogen *Candida albicans* and other *Candida* species. The Gene Ontology (GO; www.geneontology.org) is a standardized vocabulary that the *Candida* Genome Database (CGD; www.candidagenome.org) and other groups use to describe the function of gene products. To improve the breadth and accuracy of pathogenicity-related gene product descriptions and to facilitate the description of as-yet uncharacterized but potential pathogenicity-related genes in *Candida* species, CGD has undertaken a three-part project: first, the addition of terms to the Biological Process branch of the GO to improve the description of fungal-related processes; second, manual recuration of gene product annotations in CGD to use the improved GO vocabulary; and third, computational ortholog-based transfer of GO annotations from experimentally

characterized gene products using these new terms to uncharacterized orthologs in other *Candida* species. Through genome annotation and analysis, we identified candidate pathogenicity genes in seven non-albicans *Candida* species and in one additional *C. albicans* strain, WO-1. We also defined a set of the *C. albicans* genes at the intersection of biofilm formation, filamentous growth, pathogenesis and phenotypic switching and now, finger and tentacle development, of this opportunistic fungal pathogen, which provide a compelling list of candidates for further experimentation.

225. Genome sequencing of *Verticillium albo-atrum* pathotypes in order to understand wilt disease in hop production. J. Jakse[1], G. Rot[2], V. Jelen[1], S. Radisek[3], S. Mandelc[1], A. Majer[1], B. Zupan[2], B. Javornik[1]. 1) Agronomy Department, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; 2) Bioinformatics Laboratory, Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia; 3) Slovenian Institute of Hop Research and Brewing, Zalec, Slovenia.

Verticillium wilt of hop is a vascular disease caused by *V. albo-atrum*, outbreaks of the lethal strains of which threaten current hop production in Europe. Fungal isolates differ in aggressiveness and have been classified by pathogenicity tests into mild and lethal pathotypes. In general, the mild strain infection varies in intensity from year to year and rarely causes the death of the whole plant, whereas lethal strain infection causes very severe symptoms, with rapid plant withering and dieback. Lethal strains with increased virulence in hop were first reported in the UK in 1933, followed by outbreaks in Slovenia in 1997 and in Germany in 2005. Sequencing the genomes of mild and lethal *V. albo-atrum* hop isolates aimed at the dissection of the pathotype genomes, in order to provide an insight into their genomic structure, which might explain the increased virulence of the lethal strain, enable the detection of virulence-associated factors and elucidate the pathogenicity in *Verticillium spp*. Genomes of three mild and three lethal strains from three different geographic regions were sequenced by Illumina technology. The reference lethal strain, with a larger genome than the mild strains, as confirmed by flow cytometry, was sequenced using three different length libraries producing a total of 76.3 M reads. From 4.8 to 11.5 M reads were obtained for the other five strains. Additionally, 38.3 M RNA-seq reads of mild and lethal strain transcriptomes were produced for annotation of the transcribed regions. Bioinformatics analyses included *de-novo* assembly of the reference genome, followed by mapping of the other genomes for comparison of mild and lethal strains to determine specific regions of the strains. The reference genome was assembled into 715 contigs, with a total length of 33.59 Mb. Comparison of lethal versus mild strains revealed that 0.5 Mb of DNA was only present in the lethal strains. Gene prediction tools supported by RNA-seq analysis revealed 9858 gene models, 91 of which were present in the lethal unique region. Analysis of repetitive DNA based on prebuilt models masked 1.53% of the assembled genome, while *de-novo* identification of repeats masked 5.86% of the genome. The presented sequencing study established a new genomic resource for non-alfalfa *V. albo-atrum* strains and will enable their virulence to be studied.

226. Aegerolysin proteins from *Aspergillus* species. Nada Krasevec[1], Kristina Sepcic[2], Sasa Rezonja[1,2], Nina Sluga[1,2], Peter Macek[2], Gregor Anderluh[1]. 1) L11 Laboratory for Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia; 2) Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia.

Currently, Aegerolysin family (Pfam06355) comprises over 300 proteins, mostly assigned as putative hemolysins, however, their function and biological role is unknown. Some of them, i.e. aegerolysin, ostreolysin, pleurotolysin A, erylysin A from the Basidiomycota mushrooms (*Agrocybe aegerita*, *Pleurotus ostreatus* and *P. eryngei*), and their orthologues, Asp-hemolysin from the human pathogens *Aspergillus fumigatus* (Eurotiales, Ascomycota) and PA0122 (rahU) from *Pseudomonas aeruginosa* (Proteobacteria), have been characterized as lipid- or membrane-binding proteins. Aegerlysins are specifically distributed among certain fungal species belonging to both Ascomycota and Basidiomycota taxa, however, they could be also found in bacteria and plants. In 2004, it was reported that in addition to the aegerolysin component A (pleurotolysin A, PlyA), a 59 kDa component B (pleurotolysin B, PlyB) is obligatory for the observed hemolytic activity of these proteins. In contrast to aegerolysins (component A), that appear widely distributed among different organisms, initial bioinformatical search of component B homologues results in a much lower number of similar putative proteins, even more, both components combined could be found in a few of fungal species only. Joint Genome Institute (JGI) has recently sequenced eight *Aspergillus* species (*A. tubingensis*, *A. brasiliensis*, *A. acidus*, *A. glaucus*, *A. versicolor*, *A. sydowii*, *A. wentii* and *A. zonatus*) as a result of community sequencing proposal (CSP2011). The genome sequences are available at MycoCosm (JGI) (http://genome.jgi.doe.gov/programs/fungi/index.jsf) and at the Aspergillus Genome Database (AspGD) (http://www.aspgd.org/). The task of EUFGEN (EURotiales Functional GENomics consortium, http://www.eufgen.org/) is to complement these genome sequences to those already available for the *Aspergillus* species. The strains were provided by CBS-KNAW fungal biodiversity center (http://www.cbs.knaw.nl/collection/AboutCollections.aspx). Our aim is to clarify experimentally the relation between the genome context for the two components and their presumed hemolytic activity.

227. Assembly, Annotation, and Analysis of Multiple Mycorrhizal Fungal Genomes. Alan Kuo[1], Igor Grigoriev[1], Annegret Kohler[2], Francis Martin[2], Mycorrhizal Genomics Initiative (MGI) Consortium. 1) Fungal Genomics Program, DOE Joint Genome Institute, 2800 Mitchell Dr., Walnut Creek, CA, 94598 USA; 2) Lab of Excellence ARBRE, Department of Tree-Microbe Interactions, INRA-Nancy, 54280 Champenoux, France.

Mycorrhizal fungi play critical roles in host plant health, soil community structure and chemistry, and carbon and nutrient cycling, all areas of intense interest to the US Dept. of Energy (DOE) Joint Genome Institute (JGI). To this end we are building on our earlier sequencing of the Laccaria bicolor genome by partnering with INRA-Nancy and the mycorrhizal research community in the MGI to sequence and analyze dozens of mycorrhizal genomes of all Basidiomycota and Ascomycota orders and multiple ecological types (ericoid, orchid, and ectomycorrhizal). JGI has developed and deployed high-throughput sequencing techniques, and Assembly, RNASeq, and Annotation Pipelines. In 2012 alone we sequenced, assembled, and annotated 12 draft or improved genomes of mycorrhizae, and predicted ~232831 genes and ~15011 multigene families, All of this data is publicly available on JGI MycoCosm (http://jgi.doe.gov/fungi/), which provides access to both the genome data and tools with which to analyze the data. Preliminary comparisons of the current total of 14 public mycorrhizal genomes suggest that 1) short secreted proteins potentially involved in symbiosis are more enriched in some orders than in others amongst the mycorrhizal Agaricomycetes, 2) there are wide ranges of numbers of genes involved in certain functional categories, such as signal transduction and post-translational modification, and 3) novel gene families are specific to some ecological types.

228. Comparative reannotation of 21 Aspergillus genomes. Asaf A. Salamov, Robert Riley, Igor Grigoriev. DOE Joint Genome Inst, Walnut Creek, CA.

We used comparative gene modeling to reannotate 21 Aspergillus genomes from MycoCosm and AspGD.Initial automatic annotation of individual genomes may contain some errors of different nature, for example, missing genes, incorrect exon-intron structures, 'chimeras', which fuse 2 or more genes,or splitting genes into 2 or more models.The main premise behind the comparative modeling approach is that for closely related genomes most orthologous families have the same conserved gene structure. The algorithm maps all gene models predicted in all individual Aspergillus genomes to each genomes and for each locus selects among the potentially many competing models the one, which most closely resembles the orthologous genes from other genomes. This procedure is iterated until no change in gene models will be observed. For the 21 Aspergillus genomes we predicted a total of 4503 new gene models (~2% per genome), supported by comparative analysis, additionally correcting ~18% of oldgene models. This resulted in total of 4065

more genes with annotated PFAM domains(~3% increase per genome). Analysis of few genomes with transcriptomics data shows that new annotation sets also have a higher number of EST-supported splice sites at exon-intron boundaries.

229. Using the phenotypic information in the PHI-base database to explore pathogen genomes, transcriptomes and proteomes. Martin Urban[1], John Antoniw[2], Natalia Martins[3], Artem Lysenko[2], Jacek Grzebyta[2], Elzbieta Janowska-Sedja[2], Mansoor Saqi[2], Kim Hammond-Kosack[1]. 1) Plant Biology and Crop Science, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom; 2) Computational and Systems Biology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom; 3) Embrapa - Genetic Resources and Biotechnology, Brasília, Brazil.

The Pathogen-Host Interactions database (www.phi-base.org), called PHI-base, stores expertly curated molecular and biological information on genes for which the effect on pathogen-host interactions has been tested experimentally. Fungal, oomycete and bacterial pathogens which infect animal, plant, fish, insect and/or fungal hosts are included. Information is also given on the target sites of some anti-infective chemistries. This database, available since 2005, is used to analyse effectively the growing number of verified genes that mediate an organism's ability to cause disease and/or to trigger host responses. PHI-base is also used as a valuable resource for the functional annotation of novel genomes (http://phytopathdb.org), in comparative genomics studies and for the discovery of candidate targets in medically and agronomically important microbial pathogens for intervention with synthetic chemistries and natural products (fungicides). Each curated entry in PHI-base is checked by individual species experts and is supported by strong experimental evidence (e.g. gene deletion, complementation experiments) and literature references. This extensive manual curation aims to position PHI-base as a 'gold standard' for researchers in the pathogen-host biology community. Genes are annotated using controlled vocabularies (Gene Ontology terms, EC Numbers, etc.), and links to other external data sources (for example, NCBI taxonomy, EMBL and UniProt) are provided. Here we describe a significant update of PHI-base (Version 3.4) in which the data content has more than doubled. PHI-base now provides information on more than 2,200 genes described in 3000 pathogen-host interactions, which are associated with more than 106 pathogenic species. A Fusarium species case study is presented, where the database content has been used in an integrated network analysis (combining information from gene co-expression, predicted protein-protein interactions and sequence similarity) to predict proteins in *Fusarium graminearum* that may be involved in pathogenicity. This approach has identified 215 candidates including 29 proteins currently annotated as 'hypothetical'. As the content of PHI-base grows, we expect this database to be an important resource for exploring conserved and species-specific themes in pathogenicity.

230. RNA-Seq analysis reveals new gene models and alternative splicing in *Fusarium graminearum*. Chunzhao Zhao[1,2], Cees Waalwijk[1], Pierre Wit[1], Dingzhong Tang[2], Theo vanderLee[1]. 1) Wageningen-UR, Wageningen, Gelderland, Netherlands; 2) 3State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

The genome of *Fusarium graminearum* has been sequenced and annotated, but correct gene annotation remains a challenge. In addition, posttranscriptional regulations, such as alternative splicing and RNA editing, are poorly understood in *F. graminearum*. Here we took advantage of RNA-Seq to improve gene annotations and to identify alternative splicing and RNA editing in *F. graminearum*. In total 25,720,650 reads were generated from RNA-Seq. Transcripts were detected for 84% of the genes predicted by machine annotation in the BROAD database, Of these reads, 74.8% matched to exonic regions, 10.6% to untranslated regions (UTRs), 12.9% to intergenic regions and only 1.7% to intronic regions. We identified and revised 655 incorrectly predicted gene models (10% of the gene models that could be tested), including revisions of intron predictions, intron splice sites and prediction of novel introns. In addition, we identified 231 genes with two or more alternative splice variants, mostly due to intron retention. In-frame analysis showed that the majority of these alternatively spliced transcripts lead to premature termination codons, PTCs. Apart from PTC isoforms, some alternatively spliced transcripts encoding proteins with diverse lengths were identified. The effects of the diversity in the transcript length on the biological function of proteins are still unknown, but several functions including binding properties, intracellular localization, enzymatic activity or stability may be affected. Interestingly, the expression ratios between different transcript isoforms appeared to be developmentally regulated. Surprisingly, no RNA editing was identified in *F. graminearum*. Moreover, 2459 novel transcriptionally active regions (nTARs) were identified and our analysis indicates that many of these could be genes that were missed in the automated annotation. A number of representative novel gene models and alternatively spliced genes were validated by reverse transcription polymerase chain reaction and sequencing of the generated amplicons. Our results demonstrate that posttranscriptional regulation can be studied efficiently using our developed RNA-Seq analysis pipeline and may be important in adaptation of *F. graminearum* to changing environmental conditions that occur during different growth stages.

231. Comparison of transcriptome technologies in the MpkA deletion mutant of *Aspergillus fumigatus*. Clara Baldin[1,3], Sebastian Mueller[2], Marco Groth[4], Konrad Gruetzmann[5], Reinhard Guthke[2], Olaf Kniemeyer[1,3], Axel Brakhage[1,3], Vito Valiante[1]. 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, 07745 Jena, Germany; 2) Department of Systems Biology / Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, 07745 Jena, Germany; 3) Department of Microbiology and Molecular Biology, Friedrich Schiller University Jena, Beutenbergstrasse 11a, 07745 Jena, Germany; 4) Genome Analysis, Leibniz Institute for Age Research - Fritz Lipmann Institute, Beutenbergstr. 11, 07745 Jena, Germany; 5) Department of Bioinformatics, Friedrich Schiller University Jena, Ernst-Abbe-Platz 2, 07743 Jena, Germany.

RNA deep sequencing techniques are rising as powerfull strategy to analyze the transcriptome profile of different organisms. Especially, this approach will be very helpful whenever a microarray platform has not been established yet or when different platforms show low reproducibility of the generated data. In the present study, the expression profile of *Aspergillus fumigatus* has been analysed via different transcriptome analysis approaches. *A. fumigatus* is a saprophytic fungus that is emerging as one of the most important airborne fungal pathogens. The adaptation of this fungus to different environments stimulated research on the regulation of the cell-wall integrity pathway, which is mediated by the Mitogen Activated Protein Kinase (MAPK) MpkA. Previuos microarray analyses showed that MpkA is involved not only in the regulation of genes responsible for cell wall maintenance, but also in protection against reactive oxygen species, iron starvation response and secondary metabolites production (Jain *et al.*, Mol. Microbiol. 2011). Using the same strains and lab conditions, we performed a transcriptome study using RNA deep sequencing to directly compare different transcriptome analysis techniques. The RNA-seq technique was found to be more sensitive than microarray analyses giving us the possibility to gain new insight into the role of MpkA. We were able to identify a substantial number of novel transcripts, to detect new exons, untranslated regions, thousands of new splice junctions, and found evidence for widespread alternative splicing events. We could also identify a large group of genes belonging to known and unknown gene clusters, which are normally involved in secondary metabolite production. They are differentially regulated in the D*mpkA* mutant strain. Moreover, the transcriptome data were compared to proteome data. Comparison between these two biological levels contributes to a better understanding of transcript stability and of post-transcriptional regulatory mechanisms, giving a more global overview about MpkA regulatory circuits (Müller, Baldin *et al*., BMC 2012).

232. Spliceosome twintrons (“stwintrons”) revealed by fungal nuclear genomes. Michel Flipphi[1], Erzsébet Fekete[1], Claudio Scazzocchio[2], Levente Karaffa[1]. 1) Department of Biochemical Engineering, University of Debrecen, H-4010, Debrecen, Hungary; 2) Department of Microbiology, Imperial College London, London SW7 2AZ, UK.

The spliceosome is an RNA/protein complex, responsible for intron excision in eukaryotic genes. In mitochondria and plastids intron excision does not involve the spliceosome. For a class of chloroplast introns (II and III) "introns within introns” (twintrons) have been described. The removal of the internal intron is necessary for the excision of the external intron, and thus RNA maturation. Analogous structures have not been described for splicesomal introns. We have predicted four putative instances of “introns within introns” in nuclear genes of fungi. We call these “swtintrons” for “spliceosomal twin introns”. Putative stwitrons show a variable phylogenetic distribution. The presence of the internal intron predicts specific splicing intermediates. We have experimentally confirmed the existence of the predicted intermediate for the splicing of an RNA encoding a putative cyclic imidine-hydrolase of Fusarium verticillioides (Sordariomycetes, Hypocreales), where the internal intron interrupts the donor sequence between the first and second nucleotide and predicted an analogous structure for a gene encoding a sugar transporter in two Magnaportacea. In the bioDA gene (encoding an enzyme catalysing two steps of biotin biosynthesis of the Sordariales, an internal intron, predicted to interrupt a donor sequence of an intron between the second and third nucleotide has been confirmed by isolation of the splicing intermediate. In the fourth instance the putative internal intron disrupts the donor sequence between the fourth and fifth nucleotide of the 5’ sequence. In this instance, the presence of the internal intron was disproved, revealing an unsuspected case of alternative splicing.

233. NGS data revealed that the NSDA sterile mutant contains a mutation in the SCF ubiquitin ligase subunit gene, *culC*, in *Aspergillus nidulans*. Dong-Soon Oh[1], Dong-Min Han[2], Masayuki Machida[3], Kap-Hoon Han[1]. 1) Dept Pharmaceutical Engineering, Woosuk Univ, Wanju, Korea; 2) Division of Life Science, Wonkwang University, Iksan, Korea; 3) Bioproduction Research Institute, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan.

Sexual development and fruiting body production of fungi play pivotal roles in production of ascospores by meiosis as well as adaptation of various environmental changes. In a homothallic fungus *Aspergillus nidulans*, many environmental factors and genes affecting sexual development have been elucidated. One of the first and important attempts for understanding the sexual development of *A. nidulans* was isolation of NSD, BSD and ASD mutants, which are defective in the process. Among them, NSD mutants are divided into four different complementation groups, NSDA-D, and two of the mutants, NSDC and NSDD, have already been characterized about the responsible genes, *nsdC* and *nsdD* and their functions. However, *nsdA* and *nsdB* mutations are remained to be unveiled. Since classical complementation experiments were not successful, we analyzed the whole genome sequence of NSDA mutant obtained from Next Generation Sequencing (NGS) to identify the *nsdA4* mutation. As a result, we found three NSDA mutant-specific mutations and confirmed the mutations by PCR followed by sequencing analysis. One of the mutations was found in AN3939 locus which encodes SCF ubiquitin ligase subunit CulC. The mutation was G to T transversion, making D468Y amino acid residue change. Since the COP9 signalosome and ubiquitin ligase play important roles in fungal development, this mutation could be the correct *nsdA4* mutation responsible for the sterile NSDA mutant phenotype. However, since two more mutant-specific mutations were also found in NSDA, detailed genetic characterization and mutation analyses will have to be performed. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A1A4A01012864).

234. Whole genome sequencing of two *Aspergillus oryzae* strains isolated from Meju, a traditional brick of dried fermented soybean, in Korea. Dong-Soon Oh[1], Seung-Bum Hong[2], Jong-Hwa Kim[1], Goro Terai[3], Hiroko Hagiwara[3], Masayuki Machida[3], Kap-Hoon Han[1]. 1) Dept of Pharmaceutical Engineering, Woosuk Univ, Wanju, Korea; 2) Korean Agricultural Culture Collection, NIAB, Korea; 3) Bioproduction Research Institute, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan.

In Korea, various *Aspergillus oryzae*-like fungi are generally regarded as one of causal agents of Korean Meju, a soybean brick for soybean paste, fermentation. Since the fungal strain plays important roles in Japanese fermented food, *A. oryzae* type strain of Japan, RIB40, has been sequenced and analyzed in detail. Despite the importance of the *A. oryzae* strains in Korean fermented food, not many fungal strains have been isolated from fermented foods as well as Meju and the characteristics of the fungi isolated from Meju have not been elucidated so far, especially in molecular genetics and genomics level. In this study, we tried to reveal the differences between Japanese and Korean *A. oryzae* strains by characterizing the whole genome structure and their features. The whole genome sequence of two *A. oryzae*-like fungi, which were isolated from Korean Meju by Korean Agricultural Culture Collection (KACC), were obtained by Next Generation Sequencing. Comparison of the genome sequences between RIB40 and Korean isolates by using ortholog and homolog analyses revealed that, in one of the Korean isolates, about 50 kb subtelomeric region of chromosome III, where the aflatoxin gene cluster located, was deleted, suggesting that chromosome deletion have been occurred inside the genome of the same species. Not only the aflatoxin gene cluster but also the other regions were modified in the Korean isolates. Gene annotation analysis and characteristics including those in relation to closely related species *Aspergillus flavus* will be discussed.

235. Systematic analysis of the uncharacterized genes, which widely conserved among filamentous fungi, in *Aspergillus oryzae*. N. Imaru[1,2], F. Senoo[1,2], Y. Ikeda[1], S. Terado[1,2], K. Iwashita[1,2]. 1) National Research Institute of Brewing, Higashihiroshima, Hiroshima, Japan; 2) AdSM, Hiroshima Univ., Higashihiroshima, Hiroshima, Japan.

The genome sequences of *Aspergillus oryzae* revealed huge number of uncharacterized genes, which were occupied about 50% of *A. oryzae* genes. Most of these genes were widely conserved among other *Aspergillus* species and filamentous fungi, but not found in other organisms. Moreover, several genome array analysis revealed that some of these genes were highly expressed in various conditions, such as liquid or solid-state cultivations. In this work, we designated these gene as *cff* (Conserved among Filamentous fungi and Function unknown genes) genes. The analysis of the functions of these *cff*genes will be important to reveal the novel molecular mechanisms which conserved among filamentous fungi. In this context, we constructed *cff* genes disruptants library and analyzed the phenotype of these *cff* disruptants to examine the function of the genes and to identified new drug or breeding target genes. First of all, we isolated function unknown genes according to KOG category of *A. oryzae* genome database and further selected the genes that are conserved at least 7 species among 14 filamentous fungi as the *cff* candidate genes. Then we further examined several database, such as Swiss plot, AspGD etc., to verify the function-unknown then decided *cff* genes. From these *cff* genes, we performed the disruption of the highly expressed *cff* 147 genes and obtained 130 cff genes disruptants including 9 heterokaryon type disruptants. We observed the morphological phenotype of these *cff* genes disruptants on the minimal medium and natural medium using three serial powder plates, as a model assay of industrial conditions. As the result, some disruptants showed characteristic phenotypes in the hyphae growth and the conidiation. Furthermore, we examine the drug sensitivity of these disruptants using hydroxyurea, camptothecin, micafungin et. al.. As the results, significant growth inhibition was observed in some disruptants, while some disruptant shown slight drug resistant. Now we are going to examine stress responses and second metabolite productions. We will further analyses the detail

molecular function of the genes which shown significant phenotype in these analysis.

236. *Penicillium purpurogenum* degrades lignocellulose. What can we learn of this process by analyzing the genome, transcriptome and secretome of the fungus? Wladimir Mardones[1], Eduardo Callegari[2], Jaime Eyzaguirre[1]. 1) Department of Biology, Universidad Andres Bello, Santiago, Chile; 2) Sanford School of Medicine, Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD.

Penicillium purpurogenum grows on a variety of natural carbon sources and secretes to the medium numerous cellulolytic and hemicellulolytic enzymes. Although some information on the lignocellulose biodegradation process has been obtained by the study of individual enzymes, a more comprehensive approach has been attempted by analysis of the genome, transcriptome and secretome of the fungus. A genome sequence draft has been attained by means of Illumina Hi-Seq 2000 analysis followed by assembly (Allpaths-LG) and partial annotation (MAKER pipeline): 36 Mb total length, 579 scaffolds, N50 238 Kbp, 8984 genes predicted. Using the same sequencing technology and the Trinity assembler, a transcriptome of the fungus grown on sugar beet pulp (50% pectin, 20% cellulose) has been obtained. It includes 7,172 ESTs with mean length of 307 bp; 5195 ESTs were significantly identified in the genome. The secretome of the sugar beet pulp culture was analyzed by shotgun mass spectrometry (2D Nano-LC MS/MS) and 53 proteins were identified by MASCOT. An analysis of the genome draft for genes related to lignocellulose biodegradation enzymes (using dbCAN) showed 347 genes of putative CAZYmes (38 carbohydrate esterases, 245 glycosyl hydrolases, 56 glycosyl transferases, 6 polysaccharide lyases and 2 carbohydrate binding modules). The transcriptome data (using BLASTX) showed that 111 CAZy genes were transcribed. In addition, 46 putative CAZymes were identified in the secretome. Among the 46 recognized, 6 are cellulases and 19 are pectinases, directly related to the degradation of sugar beet pulp. This is the first *Penicillium* genome sequenced using next generation technology and annotated for its lignocellulose biodegradation enzyme genes. Most of the identified genes correspond to putative non-characterized enzymes. This information will be of value for a better understanding of the lignocellulose biodegradation by filamentous fungi. Support: FONDECYT 1100084; UNAB DI-61-12/R.

237. Functional genomics of lignocellulose degradation in the Basidiomycete white rot *Schizophyllum commune*. Robin A. Ohm[1], Martin Tegelaar[2], Han A. B. Wösten[2], Igor V. Grigoriev[1], Luis G. Lugones[2]. 1) US DOE Joint Genome Institute, Walnut Creek, CA, USA; 2) Department of Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Utrecht University, Utrecht, The Netherlands.

White and brown rot fungi are among the most important wood decayers in nature. Although more than 50 genomes of Basidiomycete white and brown rots have been sequenced by the Joint Genome Institute, there is still a lot to learn about how these fungi degrade the tough polymers present in wood. In particular, very little is known about how these fungi regulate the expression of genes involved in lignocellulose degradation. In Ascomycetes, several conserved transcription factors involved in regulation of complex carbon source degradation have been identified, but there are no homologs of these in Basidiomycetes. Few Basidiomycete white or brown rots are genetically amenable, hindering a functional genomics approach to the study of lignocellulose degradation. A notable exception is *Schizophyllum commune*, for which numerous genetic tools are available. *S. commune* was grown on several carbon sources (glucose, cellulose, lignin or beech wood) and gene expression was analyzed. Numerous genes are strongly up-regulated on the complex carbon sources, compared to on glucose. As expected, many of these encode CAZymes (notably glycoside hydrolase family 61) and FOLymes, but also several well conserved proteins with unknown function. Interestingly, three transcription factor genes are up-regulated during growth on complex carbon sources, suggesting they may be involved in regulating this process. These transcription factors are highly conserved in Basidiomycetes, but not in Ascomycetes. The two laccase genes of *S. commune* are very lowly expressed on complex carbon sources, suggesting that their function in lignocellulose degradation is limited. A promoter analysis of up-regulated genes reveals a conserved putative transcription factor binding site, which is also present in related fungi. Experiments to validate these findings, as well as a proteomics analysis during growth on complex carbon sources, are currently in progress.

238. Functional characterization of genes expressed in early infection stages by the phytopathogenic fungus Botrytis cinerea. J. Espino, N. Temme, A. Viefhues, B. Oeser, P. Tudzynski. Institut of Plant Biology and Biotechnology, Westf. Wilhelms University, Schlossplatz 8, 48143 Muenster, Germany.

Botrytis cinerea is a phytopathogenic fungus that causes important economic losses in the agricultural field, due to its aggressiveness and ability to produce the “grey mould disease” in more than 200 plant species. Nowadays, the main strategy of control consists in the use of fungicides, although some strains are becoming resistant to these chemicals. Therefore, the knowledge of the molecular mechanisms during host-plant interaction could be a useful tool to develop new effective treatments against this organism. In microarray studies, we have identified more than 150 genes which are expressed during the early stages of infection, but not in conidia, suggesting an important role during fungal germination and penetration. Most of these genes codify for proteins with unknown function. By means of bioinformatic analyses, transmembrane domains and signal peptides were identified in some of these proteins, suggesting a possible role in signaling pathways or as effectors in the interaction with the plant. We are currently focusing on 18 of these genes, and we validated their expression by real time PCR. In all cases the expression pattern observed in the microarrays studies could be confirmed by quantitative PCR results. Some of them showed an expression at 12 hours post inoculation even 10,000-fold compared to the expression in conidia. In order to elucidate the possible role of these genes, we have generated knock-out mutants of 9 single genes and 9 genes located in clusters. Pathogenicity studies as well as further characterization of the different deletion mutants are now in progress.

239. Regulation of biofilm formation in Candida parapsilosis. Linda Holland, Leona Connolly, Denise Lynch, Geraldine Butler. School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin, Ireland.

Candida parapsilosis is a major cause of infection in premature neonates, particularly because of its tendency to grow as biofilms on indwelling medical devices. The biofilm architecture of C. parapsilosis biofilms is substantially different to that of Candida albicans, in particular because C. parapsilosis does not make true hyphae, suggesting that the regulation of biofilm formation may also be very different. To address this question we have adapted a fusion PCR method originally developed for C. albicans to construct gene deletions in the type strain C. parapsilosis CLIB214 (1). To date, we have generated 100 homozygous deletion strains. We selected predicted protein kinase genes, transcription factors and also genes that are known to be important for biofilm formation in either C. albicans or C. parapsilosis. The collection was assayed for changes in biofilm formation using 24-well Nunc polystyrene plates and by measurement of the dry weight of mature biofilm. Eight deletion strains, efg1, czf1, mkc1, gzf3, ume6, ace2, cph2 and bcr1 have a defect in biofilm development. Only efg1 and bcr1 deletions of C. albicans have similar defects. C. albicans and C. parapsilosis therefore share some key regulators of biofilm formation, but there are also substantial differences. References: (1) Noble SM, French S, Kohn LA, Chev V, Johnson AD. Nat Genet. 2010 Jul;42(7):590-8. Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity.

240. Functional analysis of the Mps1 MAP kinase pathway in the rice blast fungus *Magnaporthe oryzae*. E. Grund[1], M.-J. Gagey[1], V. Toquin[2], R. Beffa[3], N. Poussereau[1], M.-H. Lebrun[1,4]. 1) MAP CNRS-UCB-INSA-Bayer CropScience, Lyon, France; 2) Biochemistry Dept, Bayer CropScience, Lyon, France; 3) Bayer CropScience AG, Frankfurt/Main, Germany; 4) BIOGER INRA, Thiverval-Grignon, France.

Signaling pathways are important in coordinating fungal cellular processes required for stress resistance, development and pathogenicity. The Mps1 MAP kinase pathway of *Magnaporthe oryzae* is involved in cell wall integrity, sporulation and pathogenicity. D*mps1* mutants displayed an abnormal mycelial growth (reduced aerial hyphae and melanisation), did not sporulate and were non-pathogenic on plants as reported (a). Sensitivity of *M. oryzae* to cell wall degrading enzymes (CWDE) and cell wall inhibitors (CWI) was found to be dependent on pH. Indeed, *M. oryzae* cell walls display a resistance to enzymatic degradation at pH 5, while they are sensitive at pH 6. D*mps1* loses this pH 5 induced cell wall resistance, while it is as sensitive to CWDE as wild type at pH 6. *M. oryzae* is highly resistant to calcofluor (cell wall disorganizing agent) at pH 5 (10x) compared to pH 6. D*mps1* loses this pH 5 induced calcofluor resistance, while it is as sensitive as wild type at pH 6. *M. oryzae* is more sensitive (20x) to Nikkomycin Z (chitine synthase inhibitor) at pH 5 than pH 6, while sensitivity to Aculeacin (glucan synthase inhibitor) is independent of the pH. However, D*mps1* is as sensitive as wild type to these inhibitors at both pH. We conclude that the pH 5 induced resistance of fungal cell walls to CWDE and calcofluor requires the Mps1 pathway. This also suggests that the Mps1 pathway is strongly activated at pH 5 compared to pH 6. To test this hypothesis, we are assaying the phosphorylation status of Mps1 at different pH as well as under several stress conditions and developmental stages to know when this pathway is activated. Additionally we constructed an activated allele of Mkk1, the MAPKK upstream of Mps1, placed under the control of either its own promoter (b) or the repressible *pNIA1* promoter. These transformants will be used to assess the effect of controlled activation of the Mps1 pathway on *M. oryzae* cellular functions. The different conditions of Mps1 pathway activation will be used for a comparative transcriptomic analysis of wild type and D*mps1* mutants.

(a) Xu, 2000. Fungal Genet. Biol. 31:137-152.
(b) Fujikawa *et al.*, 2009. Mol. Microbiol. 73(4):553-70.

241. A RNA-Seq directed functional genomics screen to identify novel cell wall genes in the hyphal tip of Neurospora crassa. Divya Sain, Lorena Rivera, Jason Stajich. Plant Pathology & Microbiology, University of California, Riverside, Riverside, CA.

The cell wall is one of the most important organelles of the fungal cell and differentiates pathogenic fungi from the plants and animals they infect. This makes cell wall biosynthesis an excellent target for anti-fungal drugs. To identify new targets we employed a functional genomics approach informed by gene expression patterns based on RNA-Seq of the filamentous fungus *Neurospora crassa*. The growing tips of fungal hyphae are enriched for cell wall biosynthesis activity proteins and transcripts (1-2). Based on this idea we sequenced RNA from the tip (1 hr growth) and colony interior (20 hr growth) of vegetative growing culture of *N. crassa*. 70 genes were up-regulated in the tip (at least 5 fold) and we supplemented this list with 42 tip expressed genes from a study of *N. crassa* colony development using microarrays, where mRNA transcripts in the colony tips were enriched in functional categories related to cell wall growth and morphogenesis (2). We used the *N. crassa* knockout collection (3) to identify developmental phenotypes and under chemical stress conditions to expose sensitivity in cell wall and growth defects. Almost 60 percent of the genes were found to be sensitive to cell wall stress agents, Caspofungin (cell wall integrity inhibition) and SDS (cell wall disruption) suggesting that our gene-set was enriched for genes having a cell wall defect. We tested these genes for defects in the hyper-osmolar stress (NaCl & Glycerol) and oxidative stress pathways as well as sexual development pathway. We found 20 knockout strains having defects in all or nearly all of these pathways suggesting these cell wall genes are involved in multiple pathways of growth and development of filamentous fungi. This set includes Zn-Cys transcription factors (NCU04866 & NCU04663), Glycoside Hydrolase 13 family proteins (NCU08131 & NCU08132) and genes with no annotated function (NCU04826 & NCU01254). All of these genes possess homologs in other Peziomycotina fungi. Hence our approach using gene expression selected a candidate gene-set enriched for growth processes that may be useful as targets for anti-fungal drug development against filamentous pathogenic fungi. 1) Bartnicki-Garcia & Lippman. Science 1969; 165(3890):302-4. 2) Kasuga & Glass. Euk Cell 2008; 7(9):1549-64. 3) Colot et al. PNAS 2006; 103(27):10352-7.

242. Identification of centromeres in the plant pathogen *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*). Klaas Schotanus[1], Lanelle R. Connolly[2], Kristina M. Smith[2], Michael Freitag[2], Eva H. Stukenbrock[1]. 1) MPRG Fungal Biodiversity, Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

Several plant pathogenic fungi contain small, apparently dispensable chromosomes, and in several cases pathogenicity genes have been identified on these chromosomes. The ascomycete *Zymoseptoria tritici* has up to eight dispensable chromosomes in addition to thirteen “core” chromosomes. During meiosis dispensable chromosomes are lost at elevated rates, resulting in progeny with distinct novel chromosome sets. So far little is known about the role of these chromosomes and their evolutionary dynamics. We hypothesize that loss of dispensable chromosomes during meiosis may be correlated to unstable centromeres. Thus, one goal was to identify and characterize centromeric regions on core and dispensable chromosomes to allow us to investigate the underlying genetics of chromosome instability. Both core and dispensable chromosomes in the *Z. tritici* reference isolate IPO323 have been sequenced from telomere to telomere, yielding a unique opportunity to identify the centromeric regions in the genome. We tagged the *Z. tritici* centromere-specific histone 3 (CenH3) with GFP and confirmed correct insertion by Southern analyses. We demonstrate expression of GFP-tagged CenH3 by western blot and epifluorescence microscopy. ZtCenH3-GFP was localized in discrete foci in interphase nuclei, but in contrast to other fungi (e.g., *Neurospora*, *Fusarium*, *Saccharomyces* and *Schizosaccharomyces*) there are several foci per nucleus instead of a single chromocenter. We also performed chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) on the CenH3-GFP strains. To confirm the CenH3-GFP results, we tagged two additional centromere proteins (CEN-B and CEN-S) with GFP. To assess stability of centromeres during mitosis and meiosis, we obtained evolved asexual progeny of IPO323 after 50 and 100 generations and progeny from a cross. Comparison of centromeric positions in the genome of the founder strain (IPO323) *versus* sexual and asexual progeny will allow us to infer dynamics of centromeres on core and dispensable chromosomes, and aid in our understanding of the evolutionary dynamics of dispensable chromosomes in fungal plant pathogens.

243. Loss of the RNAi pathway in VGII *Cryptococcus gattii* sheds light on the intact system in *Cryptococcus neoformans*. R Blake Billmyre, Xuying Wang, Marianna Feretzaki, Joseph Heitman. Duke University, Durham, NC.

Loss of RNAi in VGII *Cryptococcus gattii* sheds light on RNAi roles in *Cryptococcus neoformans* R. Blake Billmyre, Xuying Wang, Marianna Feretzaki, and Joseph Heitman RNAi is a broadly conserved homology-dependent silencing mechanism which functions to defend the genome by silencing transposons and viral elements. The opportunistic human pathogen *C. neoformans*, utilizes an RNAi-dependent process to robustly silence repetitive elements during the sexual cycle. Interestingly, RNAi components have been broadly lost from the VGII subtype of the closely related sister species *C. gattii*. We have taken a comparative genomics approach to compare the RNAi deficient genome of VGII *C. gattii* with the RNAi proficient genomes of VGI *C. gattii* and serotypes A and D *C. neoformans*. This approach has identified a total of fourteen gene losses or truncations of otherwise conserved genes in VGII, including three of the known canonical RNAi components. Two of the remaining eleven genes have been shown to have a role in the sex-induced silencing pathway in *C. neoformans* var. *grubii*, despite a lack of homology with previously identified RNAi components in other organisms. One of these genes *CPR2*, was previously studied in our lab as a constitutively active G-protein coupled pheromone receptor. *cpr2D* also confers a moderate defect in sex-induced silencing, but no defect in silencing during vegetative growth. Similarly, the zinc finger factor Znf3 was previously identified in our lab and here was

unexpectedly found to be important for both sex-induced and vegetative silencing. The nine remaining missing genes are being tested for roles in both sex-induced and mitotic silencing (SIS, MIS).

244. A chemical-genetic map of a human fungal meningitis pathogen. Jessica C. S. Brown[1], Benjamin VanderSluis[2], Raamesh Deshpande[2], Arielle Butts[3], Sarah Kagan[4], Itzhack Polacheck[4], Damian J. Krysan[3], Chad L. Myers[2], Hiten D. Madhani[1]. 1) Biochemistry and Biophysics, U. California, San Francisco, San Francisco, CA; 2) Computer Science and Engineering, U. Minnesota, Minneapolis, MN; 3) Pediatrics, U. Rochester Medical Center, Rochester, NY; 4) Clinical Microbiology and Immunology, Tel Aviv University, Tel Aviv, Israel.

The systematic profiling of the impact of small molecules on the growth rate of gene deletion mutants is termed chemogenomic profiling. This approach been extensively used in model organisms, primarily baker's yeast, to functionally annotate genes and to obtain insights into mode-of-action (MOA) for chemical compounds (1). Here we describe the application of systematic chemical-genetics a significant human pathogen. *Cryptococcus neoformans* is an opportunistic basidiomycetes pathogen responsible for lethal meningitis in immunocompromised patients. Current therapies are inadequate due to a paucity of drugs and a poor understanding of pathogenesis. Our laboratory previously constructed a partial gene deletion collection and used it to identify numerous genes required for infection as well as for the production of virulence factors (2). This work identified numerous novel infectivity genes, but many did not have an identifiable molecular function. We have now used chemogenomic profiling to both bridge this gap in gene annotation and to obtain insights into drug MOA. To accomplish this goal, we identified and utilized over 200 diverse chemical compounds that impact pathogen growth to create a unique phenotypic signature for ~1500 *C. neoformans* gene deletion strains. We used colony arrays, robotics, automated image analysis, and extensive data normalization algorithms to analyze several million phenotypic measurements. We used these data to identify clusters of genes and compounds with related patterns of chemical-genetic interactions. Our analysis identified virulence genes that act through related mechanisms. For example, one gene set involves a number predicted to be involved in histone modification. Members of a second set of genes are required in production of the *C. neoformans* polysaccharide capsule, a well-established virulence factor. We have also obtained new insights into the MOA of several antifungal compounds. The *Cryptococcus* chemical-genetic map will be a valuable resource for functional annotation of the genome of this meningitis pathogen, characterization of new drug targets, and the identification lead compounds for antifungal drug development.

1. Hillenmeyer et al., Science 320 (2008). 2. Liu et al., Cell 135 (2008).

245. Whole genome sequencing of high-mortality and low-mortality strains of *Cryptococcus neoformans* var. *grubii* to discover genetic determinants of virulence. Tami R. McDonald, Kirsten Nielsen. Department of Microbiology, University of Minnesota, Minneapolis, MN.

In sub-Saharan Africa, meningitis caused by the fungus *Cryptococcus neoformans* var. *grubii* is a major cause of AIDS-related mortality. To investigate the role of fungal genotype in clinical disease, we sequenced 8 genes for 503 clinical isolates of *Cryptococcus neoformans* var. *grubii*. A phylogenetic analysis of these strains demonstrated that 501 isolates were VNI strains. Haplotype network analysis revealed three major groups (BURST groups 1 - 3). Patient mortality was associated with fungal strain genotype, with strains in BURST group 3 demonstrating low mortality. Whole genome sequencing of 13 representative genotypes revealed SNPs unique to the high mortality strains, and SNPs unique to the low-mortality strains, pointing to possible targets for future gene deletion and allele swap experiments to determine the role of the genes in pathogenesis.

246. Identification of high temperature-regulated genes controlled by Sch9 through comparative transcriptome analysis in *Cryptococcus neoformans*. Dong-Hoon Yang[1], Kwang-Woo Jung[1], Jang-Won Lee[1], Min-Hee Song[1], Anna Floyd[2], Joseph Heitman[2,3], Yong-Sun Bahn[1]. 1) Biotechnology Dept, Yonsei University, Seoul, South Korea; 2) Departments of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 3) Departments of Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA.

Adaptation to temperature changes is one of crucial virulence factors for *Cryptococcus neoformans* during host infection. In the human fungal pathogen, diverse signal transduction pathways, such as Ras/Cdc24, calmodulin/calcineurin, Mpk1 and Hog1 MAPK pathways, are involved in the temperature adaptation process. In addition to the pathways, the Sch9 protein kinase has been implicated in thermotolerence of *C. neoformans*, but its regulatory mechanism remains elusive. In this study we aimed to identify Sch9-dependent or -independent temperature-regulated genes in a genome scale and to elucidate the regulatory mechanism of Sch9 in thermotolerance of *C. neoformans*. For this purpose, we performed comparative transcriptome analysis with the wild type serotype A H99 strain and *sch9D* mutant during temperature upshift from 25°C to 37°C or 40°C. The temperature upshift caused a global scale of remodeling in gene expression profiles (1872 genes, $P<0.05$) in the wild type strain of *C. neoformans*. Gene expression patterns shown during temperature shifting from 25°C to 40°C were generally similar, albeit with the different induction or repression levels, to those from 25°C to 37°C. Especially the expression levels of the genes encoding chaperone, heat shock protein, or the proteins related to ergosterol biosynthesis were differentially regulated by temperature shifting. The basal expression levels of a number of the temperature-regulated genes were modulated by Sch9 protein kinase. Interestingly the basal expression levels of the genes related to unfolded protein response (UPR) pathways including *IRE1*, *HXL1*, and *KAR2* were also regulated by Sch9 protein kinase, implying the potential link between Sch9 and the UPR pathway. Supporting this finding, the *sch9D* mutant showed increased resistance to ER stress agents, including tunicamycin and DTT. This study not only provides further insight into the regulatory mechanism of thermotolerance in *C. neoformans*, but also elucidates the regulatory mechanism of Sch9 in thermotolerence through a genome-scale identification of the Sch9-dependent genes.

247. Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay. Chiaki Hori[1,2], Kiyohiko Igarashi[1], David Hibbett[3], Bernard Henrissat[4], Masahiro Samejima[1], Dan Cullen[2]. 1) Graduate School of Agricultural and Life sciences, University of Tokyo, Tokyo, Japan; 2) Forest Products Laboratory, USDA, Madison, WI; 3) Biology Department, Clark University, Worcester, MA; 4) CNRS, Marseille, France.

Many efficient wood decay fungi belong to the Polyporales, and these can be categorized as white-rot fungi or brown-rot fungi, based on decay patterns. White-rot fungi degrade cell wall polysaccharides such as cellulose and hemicellulose as well as the more recalcitrant phenylpropanoid polymer, lignin. In contrast, brown-rot fungi depolymerize the polysaccharides but the modified lignin remains in the wood. Comparative analysis of white- and brown-rot gene repertoires and expression profiles have revealed substantial variation but considerable uncertainty persists with respect to precise mechanisms. Addressing this issue, we performed genome-wide analysis of carbohydrate-active enzymes (CAZy) and some oxidative enzymes related to polysaccharides degradation in eleven white- and brown-rot fungi. This analysis included classifying and enumerating genes from three recently sequenced polyporales *Bjerkandera adusta*, *Ganoderma sp.* and *Phlebia brevispora*. Furthermore, comparative secretomic analysis of seven Polyporales grown on wood culture were conducted. Summarizing, the average number of genes coding CAZy in the genomes of white-rot fungi was 373, significantly more than the 283 observed in brown-rot fungi. Notably, white-rot fungi have genes encoding cellulase and hemicellulase such as those belonging to glycoside hydrolase (GH) families 6, 7, 9 and 74, whereas these are lacking in genomes of brown-rot polyporales. White-rot genes encoding oxidative enzymes potentially related to cellulose degradation such as cellobiose dehydrogenase (CDH), polysaccharides monooxygenase (PMO, formerly GH61), cytochrome b562 with cellulose-

binding module, are also increased relative to brown-rot fungi. Indeed, secretomic analysis identified GH6, GH7, CDH and PMO peptides only in white-rot fungi. Overall, these results show that, relative to brown rot fungi, white rot polyporales maintain greater enzymatic diversity supporting lignocellulose attack.

248. Genomic context and distribution of effector genes in *Fusarium oxysporum*. Sarah Maria Schmidt[1], Peter van Dam[1], Petra M. Houterman[1], Ines Schreiver[2], Lisong Ma[1], Stephan Amyotte[3], Biju Chellappan[1], Sjef Boeren[4], Frank L.W. Takken[1], Martijn Rep[1]. 1) Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands; 2) Fachgebiet Medizinische Biotechnologie, Institut für Biotechnologie, Technische Universität Berlin, Gustav-Meyer-Allee 25, Germany; 3) Department of Plant Pathology, University of Kentucky, 201F Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312, USA; 4) Laboratory for Biochemistry, Wageningen University, Dreijenlaan 3, 6703HA, Wageningen, the Netherlands.

Strains of the *Fusarium oxysporum* species complex (FOSC) are able to infect a wide range of mono- and dicotyledonous plants. Based on the host specificity of individual strains, the FOSC is divided into various *formae speciales*. All strains share a common core genome and possess additional lineage-specific (LS) chromosomes. The fungus secretes effector proteins into the host vascular system that presumably manipulate the host to promote infection. In the tomato pathogen *F. oxysporum* f. sp. *lycopersici* (*Fol*) these effectors are encoded by *SIX* (Secreted In Xylem) genes. Interestingly, all *SIX* genes are present on a single LS chromosome that can be transferred horizontally to a previously non-pathogenic *Fo* strain, resulting in gain of pathogenicity towards tomato. Upon close inspection of this tomato pathogenicity chromosome we discovered that a non-autonomous miniature transposable element (mite) is present in the promoters of all *SIX* genes. Promoter deletion analysis at two different *SIX* gene loci did not reveal a direct role of the mite for *SIX* gene expression. However, we were able to use this genomic signature to predict novel effector gene candidates in the *Fol* genome. Expression of several of these novel candidates during infection was confirmed by mass spectroscopic analysis of the xylem sap of *Fol*-infected tomato plants. We also discovered a small reservoir of 'silent' effector genes that are not expressed during infection. Next, we used our method to predict effector gene candidates in the genomes of several other *formae speciales* and developed a more global picture of the effector gene complement in the FOSC. Effector genes in *Fo* consistently reside in repeat-rich enviroments. Some strains contain 2 or 3 paralogs of an effector gene. Additionally, many genomes feature truncated effector gene homologs. Overall, the effector gene distribution among different *formae speciales* is patchy, and there is no unique set of effectors that is common to all plant pathogenic strains of the FOSC.

249. Whole genome sequencing reveals new links between diverse plant pathogens; an expanded *AvrLm6*-like gene family in *Venturia* species. Jason Shiller[1], Angela van de Wouw[2], Dan Jones[1], Joanna Bowen[3], Carl Mesarich[3], Matthew Templeton[3], Kim Plummer[1]. 1) La Trobe University, Melbourne, Australia; 2) University of Melbourne, Melbourne, Australia; 3) Plant and Food Research, Auckland, New Zealand.

Venturia inaequalis and *V. pirina* are hemi-biotrophic fungi that cause apple scab and pear scab, respectively. These diseases cause significant losses to growers worldwide. In some cases, scab is controlled with resistant cultivars, but fungicides are more commonly used. Resistance to scab follows the gene-for-gene model, whereby a gene coding for a resistance protein in the host will have a cognate gene coding for an avirulence protein (or effector) in the fungus. No *Venturia* effectors have been characterised to date, but work is underway to identify effectors from the whole genome sequences and secreted proteins of *V. inaequalis* and *V. pirina*. Whole genome sequencing of *Venturia inaequalis* and *V. pirina* has revealed predicted proteins with some sequence similarity to AvrLm6, a *Leptosphaeria maculans* effector that triggers resistance in *Rlm6* canola. The mechanism of action of AvrLm6 is unknown. Until recently, AvrLm6 was thought to be unique to *L. maculans*, with orthologues absent, even in closely related species. *AvrLm6*-like genes from *Venturia spp* whose genomes are sequenced form large families containing up to 30 members. We have also identified orthologues in *F. oxysporum* (Fo5176) and *C. higginsianum* from public database searches. Gene expansions have also been observed for other effector-like genes in the Venturia genomes. The AvrLm6-like predicted protein from *V. inaequalis*, with the highest sequence identity to AvrLm6, was unable to trigger a resistance response in *Rlm6* canola. However, this does not preclude the AvrLm6-like proteins from being functionally active in the *Malus*-*Venturia* pathosystem. Transcriptome analyses (RNA-seq) of *in planta* and *in vitro* samples of *V. inaequalis* have revealed that a number of *AvrLm6*-like genes are up-regulated during infection (compared to growth *in vitro*). These results were confirmed with qRT-PCR. The most highly up-regulated predicted protein, ALVi_149, was tagged with YFP. YFP expression was observed only in the sub-cuticular stromata (specialised, biotrophic infection structures). RNA silencing is currently underway to determine the role of ALVi_149 in pathogenicity of *V. inaequalis*. The major question that remains is; what purpose do these genes serve for these diverse fungi and what is driving the gene expansions in *Venturia spp*?

250. Oömycetes Protein Array Project. Samantha Taylor[1], Regina Hanlon[2], Mandy Wilson[2], Jean Peccoud[2], Brett Tyler[1]. 1) Oregon State University, Corvallis, OR; 2) Virginia Tech, Blacksburg, VA.

Oömycetes are eukaryotes that outwardly resemble fungi, but are related to brown and golden-brown algae. The most destructive oömycete genus is Phytophthora, with over 80 species that collectively attack a wide range of plant species, causing damage to crops that is estimated in billions of dollars annually in the US. The goal of the Oömycetes Protein Array Project is to generate a collection of cloned proteins from 1440 predicted oömycete effector sequences, to use Gateway® technology to facilitate the easy transfer of clones into expression vectors, and to make the resulting clones available to the scientific community for further research. When the project is over, the final collection should include 390 clones from P. sojae, 550 clones from P. infestans, 370 clones from P. ramorum, and 130 clones from H. arabidopsidis.

251. Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. Ronnie de Jonge[1,2], Melvin Bolton[3], Anja Kombrink[1], Koste Yadeta[1], Grardy van den Berg[1], Bart Thomma[1]. 1) Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; 2) VIB Department of Plant Systems Biology, Ghent University, Bioinformatics and Evolutionary Genomics Division, Technologiepark 927, B-9052 Gent, Belgium; 3) United States Department of Agriculture, Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND 58102-2765, United States.

Sexual recombination drives genetic diversity in eukaryotic genomes, and fosters adaptation to novel environmental challenges. Although strictly asexual microorganisms are often considered as evolutionary dead ends, they comprise many devastating plant pathogens. Presently, it remains unknown how such asexual pathogens generate the genetic variation that is required for quick adaptation and evolution in the arms race with their hosts. Here we show that extensive chromosomal rearrangements in the strictly asexual plant pathogenic fungus *Verticillium dahliae* establish highly dynamic 'plastic' genomic regions that act as a source for genetic variation to mediate aggressiveness. We show that these plastic regions are greatly enriched for *in planta*-expressed effector genes, encoding secreted proteins that enable host colonization including the previously identified race 1-specific effector Ave1 that activates Ve1-mediated resistance in tomato. The plastic regions occur at the flanks of chromosomal breakpoints and are enriched for repetitive sequence elements, especially retrotransposons. Our results demonstrate that asexual pathogens may evolve by prompting chromosomal rearrangements, enabling

rapid development of novel effector genes. Likely, chromosomal reshuffling is a general mechanism for adaptation in asexually propagating organisms.

252. Genomic census of transmembrane proteins of the marine fungus, Corollospora maritima. Derek Johnson, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR.

The class Sordariomycetes (Ascomycota) presents a model phylogenetic system to study the genomic evolution of marine fungi, as there have been at least four major independent transitions from terrestrial to marine environments. These marine fungi have adapted to an environment which requires increased control of the movement of water between the cell and the environment due to osmosis. Given this, the greatest adaptive pressure may be found working at the interface of the fungal cell with the environment in the form of transmembrane proteins and in biochemical pathways dealing in osmoregulation. We have initiated a comparative, phylogenomic study of the independent lineages of marine Sordariomycetes with the goal of determining what pathways have been modified in the transition to the marine environment. Here we present preliminary data of the draft genome of the marine fungus Corollospora maritima, which is a member of the largest family of marine fungi, Halospheriaceae (Microascales). The draft genome of C. maritima is similar to other Sordariomycetes (37 MB, 10269 predicted gene models) but is characterized by an increased number of transmembrane proteins; both in raw number (2017), and as a percentage of protein coding genes (19.64%), than non-marine members of the Sordariomycetes. Thus, it is hypothesized that C. maritima has expanded osmoregulatory protein families and adapted novel transmembrane proteins as a consequence of the transition to the marine environment. Transmembrane proteins belonging to C. maritima along with select species of fungi and Eukaryotes were predicted via hidden markov modeling using the program TMHMM and clustered into orthologous groups using Ortho-MCL. Putative protein identities (e.g., aquaporins, aquaglyceroporins, osmosensors and the sodium efflux ENA ATPases) were assigned to each cluster using the BLAST tool comparing orthologous protein cluster identities to proteins of the model organisms Neurospora crassa and Saccharomyces cerevisiae. Expansions and contractions of transmembrane proteins will be presented in a phylogenetic context, and more complex patterns of evolution, such as more ancient lineage sorting vs. lineage specific expansions, will be tested for each orthologous group of proteins using gene tree/species tree reconciliation analyses.

253. Fungal Calcium Signaling Database (FCSD). Venkatesh Moktali, Bongsoo Park, Seogchan Kang. Penn State University, University Park, PA 16802, USA.

Calcium probably is one of the most versatile elements in biological systems. It serves as a pivotal signal in controlling diverse cellular and developmental processes to ensure the healthy functioning of every organism ranging from microbes to humans. The mechanism of translating external stimuli to specific cellular and developmental responses via changes in calcium ions plays an essential role in the plant-microbe and microbe-environmental interactions. Accordingly, many genes of the calcium-signaling pathway have been found to be virulence factors of fungal pathogens. How this simple and ubiquitous ion has evolved to control so many processes is one of the central questions in biology with many practical implications. Rapid advances in genome sequencing of many fungal and oomycete species have uncovered conserved core calcium signaling genes, as well as lineage-specific features. To support systematic studies on this evolutionary variability in fungi and oomycetes and the functional roles of individual genes, we built the Fungal Calcium Signaling Database (FCSD; http://fcsd.ifungi.org/), an online platform that categorizes and annotates key calcium signaling proteins from more than 120 published fungal and oomycete genomes. The database also archives experimental results from studies on mutants of calcium signaling genes and resulting calcium signatures in both video and picture formats. The calcium signaling genes in FCSD are divided into five major groups namely, calcium-permeable channels, calcium pumps, calcium exchanger/antiporter, calcium signaling regulators, and calcium-binding proteins. Comparison of calcium signaling machineries between fungi and oomycetes has been conducted to identify evolutionary changes that have shaped up this signaling pathway in these kingdoms. The FCSD will greatly support the fungal community in studying and understanding calcium signaling.

254. Evolutionary genomic analysis of cytochrome P450 proteins in the subphyla Pezizomycotina. Venkatesh Moktali, Seogchan Kang. The Pennsylvania State University, University Park, PA 16802.

The subphylum Pezizomycotina presents a vast diversity of ecological niches and biochemical processes observed in fungal subphyla. Changes in members of the cytochrome P450 (CYP) superfamily appear to have played key roles in fungal niche adaption and evolution. Availability of genomic data from many species in this subphylum has enabled comprehensive phylogenomic studies to understand the taxon-specific genetic changes that potentially underpin the observed functional and ecological diversity. CYPs from 53 Pezizomycotina species were analyzed to study the gene birth and death patterns at the genus level. This analysis revealed niche- and class-specific CYP family expansions and contractions. Putative metabolic functions were assigned to individual CYPs in each species based on sequence similarity to functionally characterized CYP proteins. Also, pathogenic Pezizomycotina fungi were divided into three classes (hemibiotrophs, obligate biotrophs and necrotrophs) to identify CYP family expansions and innovations potentially associated with these classes. Large losses in CYP families were observed among obligate biotrophs whereas hemibiotrophs and necrotrophs showed gene gains as well as functional innovation in the form of species-specific CYP families. Examination of the classes/divisions within Pezizomycotina suggested a number of independent losses and gains in CYP families. These findings shall be presented in the poster.

255. Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis. Stephen J. Mondo, Teresa E. Pawlowska. Plant Pathology, Cornell University, Ithaca, NY.

Many eukaryotes interact with heritable endobacteria to satisfy diverse metabolic needs. Of the characterized fungal-bacterial endosymbioses, the association between Gigasporaceae (Glomeromycota) and Ca. Glomeribacter is one of the best described. Glomeribacter is a member of the Burkholderia lineage of b-proteobacteria, and was shown previously to represent one of the few cases of an ancient, long-term non-essential endosymbiont. In order to further explore what adaptations have taken place to shape this unique bacterial lifestyle, we have sequenced three Glomeribacter genomes and developed a computational pipeline to compare across bacteria engaging in different lifestyles using genome wide patterns of mutation accumulation. We used PAML to identify gene orthologs that exhibited both over-accumulation and under-accumulation of amino acid substitutions and then used these data to compare across taxa at the level of functional gene categories. We found that bacteria can be grouped by lifestyle using this approach. Glomeribacter, as expected, appears most similar to other potentially long-term non-essential endosymbionts. Therefore, we were able to exploit the differences in mutation accumulation patterns between these taxa to identify processes, which may be relevant within the particular interaction between Glomeribacter and its host. While several of these processes, including vitamin synthesis and amino acid transport, have been identified previously, we additionally discovered features related to lipid biosynthesis and energy metabolism to be of potential importance for this symbiosis. Interestingly, genes exhibiting an under-accumulation of nonsynonymous substitutions (indicative of purifying selection) in Glomeribacter tend to be involved in recombination, cell division, and ribosome maintenance. While these processes are typically fast evolving in endosymbiotic organisms, they may represent features that increase the stability of Glomeribacter in their fungal host population and increase their resilience to genetic drift. We speculate that these processes are unique to the Glomeribacter-Glomeromycota symbiosis and could partially explain why Glomeribacter has been successful as a non-essential endosymbiont for over 400 million years.

256. The Unique family of Telomere-Linked Helicases in Fungi. Olga Novikova[1,2], Mark Farman[2]. 1) Department of Biological Sciences, University at Albany, Albany, NY; 2) Department of Plant Pathology, University of Kentucky, Lexington, KY.

Subtelomeres and telomeres are highly dynamic regions of eukaryotic chromosomes and their maintenance is crucial for cellular function. Several helicases are known to be involved in maintenance of telomere integrity, e.g. RecQ-like helicases such as human BLM or WRN helicases. Curiously, RecQ-like helicase genes are found in very close proximity to telomeres in several fungal species. In the present study we performed comprehensive survey of these Telomere-Linked Helicases (TLHs) in 101 fully-sequenced fungal genomes. The TLHs were widely yet sporadically distributed among fungal species being present in 46 species belonging to all investigated groups except Zygomycetes. The TLHs were also unique to fungi. Many of the TLH genes were found either next to telomeres or at the ends of contigs, providing indirect evidence that they are telomere associated. To date, the TLH gene families are the only examples where the chromosomal positions of member genes are absolutely conserved across a kingdom. Despite the seemingly conservative positions of the TLH genes on the chromosomes, the genes themselves are clearly not well conserved because they were found in only in half of the fungal genomes surveyed. The TLHs were highly divergent from one another and demonstrated complex evolutionary histories that reflect recurrent cycles of telomere crisis and recovery. Their telomere association leads us to hypothesize that the TLHs are involved either in telomere maintenance, or in the recovery processes associated with telomere crisis.

257. Evolution of proteins containing intein- and Hedgehog-like Vint domains in Fungi. Olga Novikova, Marlene Belfort. Department of Biological Sciences and RNA Institute, University at Albany, Albany, NY.

Inteins are protein sequences that autocatalytically splice themselves out of the protein precursors - analogous to introns - and ligate the flanking regions into a functional protein. Intein-containing genes are present in all three kingdoms of life. Moreover, it was shown that the C-terminal domain of eukaryotic Hedgehog (Hh) proteins has sequence similarity to inteins. The Hh pathway is one of the fundamental signal transduction pathways in animal development and is also involved in stem-cell maintenance and carcinogenesis. Two distinct domains can be found in Hh - the N-terminal 'Hedge' domain (HhN), and the intein-like C-terminal 'Hog' or 'Hint' domain (HhC). The hedgehog pathway is absent from Fungi. However, other families of proteins were found containing Hog/Hint-like domains. Representatives from one of these families carry the von Willebrand factor type A (vWA) domain in addition to the Hog/Hint-like domain. These domains are called Vint (von Willebrand Hint-like). Vint-containing proteins were initially reported for plants, fungi and some metazoa. We explored the diversity of Vint-containing proteins, their distribution and evolutionary history in fungi. Vint-containing proteins are widely distributed among fungal lineages; however, they are absent from some of the fungal species and entire fungal groups (e.g. Saccharomyces). Based on the evolutionary pattern, we propose a modular model of the evolution for Vint-containing proteins. While the vWA domain seems to be the core functional unit, an additional domain, the U-box, is a recent acquisition. The vWA and U-box combination was found exclusively in fungi. The Vint domain is highly conserved and is likely under purifying selection. The functional role of Vint-containing proteins and the Vint domain in particular is the subject for further studies. This research has been supported by NIH grant GM44844.

258. The genome and development-dependent transcriptome of *Pyronema confluens*: a window into fungal evolution. Stefanie Traeger[1], Jason Stajich[2], Stefanie Pöggeler[3], Minou Nowrousian[1]. 1) Department of General & Molecular Botany, Ruhr University Bochum, 44780 Bochum, Germany; 2) Department of Plant Pathology and Microbiology, University of California Riverside, CA 92521, USA; 3) Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August University, 37077 Göttingen, Germany.

In the last decade, genomes of many filamentous ascomycetes have been sequenced and are invaluable for the analysis of the evolution of species and for understanding their physiological and morphological properties. However, while there are at least ten genome sequences available for each of the more derived groups of filamentous ascomycetes (Sordariomycetes, Leotiomycetes, Eurotiomycetes, Dothideomycetes), only one genome from the basal group of Pezizomycetes has been sequenced, namely that the black truffle, a fungus with a specialized life-style and fruiting body. Therefore, we sequenced the genome and transcriptome of the Pezizomycete *Pyronema confluens*, a saprobe with typical apothecia as fruiting bodies. The genome was assembled from a combination of Roche/454 and Illumina/Solexa reads. It has a size of 50 Mb, and a predicted 13369 protein-coding genes. *P. confluens* is homothallic, and we found two *MAT* loci that are not fused or in close proximity, and encode an alpha domain and an HMG domain transcription factor. Only the *MAT1-2* locus is flanked by the conserved *apn2* gene, whereas both *MAT* loci are flanked by a pair of paralogous genes not found in this location in other ascomycetes. Thus, the *P. confluens MAT* loci might reflect an evolutionary transition state on the way towards the relatively conserved genomic arrangement of *MAT* loci in higher ascomycetes. Sexual development in *P. confluens* is light-dependent, and qRTPCR analyses of predicted photoreceptor genes showed that all are upregulated by light. Fruiting body formation is stimulated only by part of the visible spectrum, and we are currently investigating the effect of different wavelengths on development and gene expression. For RNA-seq analysis, we used three conditions: growth in the light, and two different conditions allowing only vegetative growth. We analyzed expression levels for genes with different degrees of evolutionary conservation to find out if genes with different lineage-specificities are preferentially expressed under any of the conditions investigated. Interestingly, the highest percentage of genes upregulated during sexual development is found among the *P. confluens* orphan genes. This might indicate that, similar to the situation in animals, genes associated with sexual reproduction evolve more rapidly than genes with other functions.

259. Genome and transcriptome analysis of the mycoparasite *Clonostachys rosea*. Kristiina Nygren[1], Mikael Brandström Durling[1], Chatchai Kosawang[2], Dan Funck Jensen[1], Magnus Karlsson[1]. 1) Forest Mycology and Plant Pathology, Swedish Univesity of Agricultural Sciences, Uppsala, Sweden; 2) Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark.

The ascomycete *Clonostachys rosea* is an efficient antagonist against a range of fungal plant pathogens, presumably as a consequence from its mycoparasitic lifestyle. *C. rosea* is therefore used as a biological control agent against pathogens threatening agricultural crops. Still, very little is known about the mechanisms behind the mycoparasitism in *C. rosea*. By comparative genomics using *C. rosea* and publically available genome sequences from closely related species exhibiting different lifestyles we aim at exploring evolution of genes important for the transition into a mycoparasitic lifestyle. And by analyzing the transcriptome during interactions with different fungal plant pathogens we intend to get a deeper understanding on the gene expression of secreted enzymes during initial attack but also to study potential specialization towards specific fungal prey species. For these purposes we have sequenced the genome of *C. rosea* strain IK726. In addition we have performed an RNAseq study to investigate the gene expression during interactions with the two plant pathogenes *Botrytis cinerea* and *Fusarium graminearum*. Our draft genome of *C. rosea* (strain IK726) reveals that the species has a larger genome than it's closest sequenced relatives (58 Mb, which is 40 - 75 % larger than the 7 closest sequenced species). Preliminary comparative genome analyses indicate that the mycoparasitic function of *C. rosea* differs from earlier findings in mycoparasites of the closely related genus *Trichoderma*. For example, in *C. rosea* we find a significant expansion of ABC-transporter genes.

260. The mitochondrial genomes of *Fusarium circinatum*, *F. verticillioides* and *F. fujikuroi* are unexpectedly similar. G Fourie[1], N.A. van der Merwe[2], B.D. Wingfield[2], B. Tudzynski[3], M.J. Wingfield[1], E.T. Steenkamp[1]. 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. Pretoria, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 3) University of Munster, Germany.

The *Gibberella fujikuroi* species complex consists of species that are of considerable agricultural, medical and veterinary importance. Nevertheless, many of the relationships among species in this complex remain largely unresolved, irrespective of the markers employed. In this study, we considered the feasibility of using mitochondrial genes to resolve the higher level evolutionary relationships of the species in the complex. Because there is limited information available regarding the structure and evolution of mitochondrial genomes in the *G. fujikuroi* species complex, we fully characterized the mitochondrial genomes of the representative species; *Fusarium circinatum*, *F. verticillioides* and *F. fujikuroi*. Overall, the mitochondrial genomes of the three species displayed a high degree of synteny, with all the genes in identical order and orientation. This similarity also extended to the intergenic regions, as well as introns that share similar positions within genes. The results show genome similarity beyond the expected characters common to the mitochondrial genomes of the Sordariomycetes. The intergenic regions and introns generally contributed significantly to the size differences and diversity observed among these genomes. Phylogenetic analysis of the concatenated protein-coding data set separated members of the *G. fujikuroi* complex from other *Fusarium* species. The individual mitochondrial gene trees did not always support the phylogeny of the concatenated data set and at least six distinct phylogenetic trees were recovered. This incongruence could arise from biased selection on some genes or recombination among mitochondrial genomes, potentially linked to a hybridization event. The results suggest that using individual genes for phylogenetic inference could mask the true relationships between species in this complex.

261. Comparative pathogenomics: next generation dissection of mechanisms of pathogenesis on plants. Donald M Gardiner[1], Jana Sperschneider[3], Paula Moolhuijzen[4], Matthew Bellgard[4], Kemal Kazan[1], Jen Taylor[2], John Manners[2]. 1) Plant Industry, CSIRO, St Lucia, Queensland, Australia; 2) Plant Industry, CSIRO, Canberra, ACT, Australia; 3) Plant Industry, CSIRO, Perth, WA, Australia; 4) Centre for Comparative Genomics Murdoch University Perth, Western Australia, 6150.

Comparative analyses between fungal plant pathogens that share a common host have revealed important mechanisms of virulence in a number of different systems. We have an interest in understanding how Fusarium pathogens of wheat and barley cause head blight and crown rot diseases on these hosts. With modern genome sequencing technologies the power to undertake comparative analyses to assist in the understanding of key molecular mechanisms involved in pathogenesis has undoubtedly increased. We have recently sequenced an additional seven Fusarium isolates that are associated with wheat including isolates of *F. equiseti*, *F acuminatum*, *F. culmorum* and *F. pseudograminearum* and are using these in comparative analyses. Sequence based homology searching between limited numbers of selected species have been particularly powerful in identifying signatures of horizontal transfer between phylogenetically diverse species which have been an important force in the evolution of virulence and examples of these that we have shown to be involved in virulence will be discussed. We are also developing methodologies to predict genes important in virulence that consider more remote homologies between species that may represent structural and/or functional conservation which also consider phylogenetic distributions and enrichment in species with particular lifestyles or shared plant hosts.

262. Characterisation of *stuA* homologue in *Fusarium culmorum*. Matias Pasquali[1], Francesca Spanu[2], Virgilio Balmas[2], Barbara Scherm[2], Kim Hammond Kosack[3], Lucien Hoffmann[1], Marco Beyer[1], Quirico Migheli[2,4]. 1) Environment and Agrobiotechnology Dept, CRP GABRIEL LIPPMANN, Belvaux, Luxembourg; 2) Dipartimento di Agraria - Sezione di Patologia vegetale ed entomologia and Unità di ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy; 3) Wheat Pathogenomics, Department of Plant Biology and Crop Sciences , Rothamsted Research, Harpenden, Herts AL5 2JQ, UK; 4) Centro interdisciplinare per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy.

Fusarium culmorum is one of the most harmful pathogens of durum wheat and the causal agent of foot and root rot (FRR) disease. *F. culmorum* produces different trichothecene mycotoxins that are involved in the pathogenic process. The role of the gene *FcStuA*, a stuA ortholog protein with an APSES domain sharing 98.5% homology to the FgStuA protein (FGSG10129), was determined by functional characterisation of deletion mutants obtained from two *F. culmorum* wild-type strains, namely FcUK99 (a highly pathogenic trichothecene producer) and Fc233B (unable to produce toxin and with a mild pathogenic ability). The *DFcStuA* mutants originating from both strains showed common phenotypic characters including stunted vegetative growth, loss of mycelium hydrophobicity, altered pigmentation, decreased production of polygalacturonases, altered and reduced conidiation, delayed spore germination patterns and complete loss of pathogenicity towards wheat stem base/root tissue. Toxin production in mutants originating from FcUK99 strain was significantly decreased *in vitro* to 5% of the original production. Moreover, both sets of mutants were unable to colonise non-cereal plant tissues, i.e. apple and tomato fruits and potato tubers. No differences between mutants, ectopic and wild-type strains were observed concerning the level of resistance towards four fungicides belonging to three classes, the demethylase inhibitors epoxiconazole and tebuconzole, the succinate dehydrogenase inhibitor isopyrazam and the cytochrome bc1 inhibitor trifloxystrobin. *StuA* is a global regulator in *F. culmorum* and is a potential target for novel fungistatic / fungicidal molecules.

263. Comparative analysis of noncoding sequences in the Gibberella fujikuroi species complex. Christian Sieber[1], Ulrich Güldener[1], Martin Münsterkötter[1], Karsten Suhre[1,2]. 1) Helmholtz-Zentrum München, Neuherberg, Bayern, GermanyHelmholtz Zentrum München, German Research Center for Environmental Health, Institute of Bioinformatics and Systems Biology, 85764 Neuherberg, Germany; 2) Department of Physiology and Biophysics, Weill Cornell Medical College, Education City, Qatar.

Initially genome analysis methods focused on the coding part and its resulting proteins, therefore the role of noncoding DNA features was widely unregarded for a long time. Due to the luxuriance of new sequencing technologies a plurality of genome sequences are now available and many different repeat families could been identified already, which exhibit a diversity of functions such as mRNA stabilization or the control of translation (Khemici 2004, Espeli 2001). Interspersed repeats account for a considerable amount of noncoding sequence in fungal genomes. The proportion of repetitive elements and the compositions of repeat families differ from species to species whereas little is known about their origin and impact on the genomic functionality. The availability of new Fusarium genome sequences facilitate an extensive comparative approach across the Gibberella fujikuroi species complex. Besides known transposable elements and satellite repeats, a diversity of previously unknown interspersed repeat families are prominent in F.fujikuroi and closely related species. While some of them are distributed through the whole complex, others can exclusively be found in only one genome. Interestingly the predicted secondary structures of the elements exhibit a stable fold in terms of free energy and complementary base pairing. Moreover gene-chip and RNA-seq experiments reveal that some elements are part of the transcriptome and still seem to propagate further in the genome. Main questions are: What influence do interspersed repeats have on genome structure and the speciation process of fungi? How can repeats contribute to host-pathogen

interaction and specification?

264. Virulence of *Fusarium circinatum* on *Pinus* species. S. L. Slinski[1,2], B. D. Wingfield[1], T. R. Gordon[2]. 1) Genetics, University of Pretoria, Pretoria, Gauteng, South Africa; 2) Plant Pathology, University of California, Davis, California.

Fusarium circinatum causes pitch canker, an important disease of *Pinus* species worldwide. Little is know of the genetic determinants of virulence in this pathogen although virulence-related genes have been identified in other *Fusarium* species. The purpose of this work was to assess the heritability of virulence in *F. circinatum* and to identify genomic regions associated with virulence. Virulence was altered through a series of sibling crosses pushing the populations towards high or low virulence. Crossing high and low virulence parents from the F_3-High and F_3-Low generations, respectively, generated progeny (HL-F_1) with a nearly continuous distribution of virulence phenotypes. One hundred progeny were evaluated for polymorphic markers segregating for virulence by AFLP-PCR. Four markers were found to have a strong association with the long lesion length phenotypes. The AFLP-PCRs containing the markers were sequenced using Illumina HiSeq technology and the genomic regions associated with virulence were located on the *F. circinatum* genome. These regions are being studied to determine the changes resulting in loss of virulence.

265. Understanding the remodeling of the wheat grain genome expression during infection, a gate to get new insights on the molecular cross-talk controlling the development of the interaction between the wheat and *Fusarium graminearum*.. Chetouhi Cherif[1,2], Bonhomme Ludovic[1,2], Cambon Florence[1,2], Lecomte philippe[1,2], Biron G-David[3], Langin Thierry[1,2]. 1) INRA, UMR 1095 GDEC, F-63039 Clermont-Ferrand cedex 2, France; 2) UBP, UMR 1095 GDEC, F-63100 Clermont-Ferrand, France; 3) CNRS-UBP 6023 LMGE, F-63171 Aubière, France.

Despite numerous progresses in understanding the molecular plant defences against pathogens, the molecular mechanisms used by a fungal pathogen to counter the plant defences and to optimize the host cell environment for fungal growth remain largely unknown. One of the main goals of our group is to better understand resistance and susceptibility mechanisms in wheat to *Fusarium graminearum*. This fungal pathogen represents the main causal agent of Fusarium head blight (FHB), an important worldwide disease in wheat reducing grain yield and quality. Contamination of grain by trichothecene mycotoxins produced by F. graminearum during infection is the primary causes of reduced grain quality. Until now, very little is known on the molecular cross-talk between this fungus and its host during a compatible interaction. In this study, we characterize the impact of *F. graminearum* infection on the wheat cellular processes and identify wheat genes, so called susceptibility genes (S-gene), required for disease development. Until now, very little is known on the transcriptional changes induced by *F. graminearum* in a susceptible wheat cultivar. A whole genome expression analysis was performed on the French susceptible wheat cultivar Récital challenged with a pathogenic and mycotoxigen *F. graminearum* strain. Using a microarray analysis, we have identified 1,453 differentially expressed genes while proteome comparative analysis showed 80 differentially regulated proteins between healthy and Fusarium-damaged kernels at different development stages of the grain (flowering at 450 °Cd). These disease-associated genes and proteins belong to three main functional groups including (i) plant defense, (ii) primary, secondary and energy metabolism and (iii) regulation and signaling. These results demonstrate that the *F. graminearum* infection strategy associates (i) suppression of plant defense, and (ii) subtle changes in nutrient availability related-processes. These preliminary results strongly suggest that *F. graminearum* manipulates the functioning of wheat kernel cells to optimize its nutrition, and therefore that the disease susceptibility of wheat relies on a parasite manipulation by this pathogenic fungi. This is the first exhaustive study of the molecular mechanisms associated with FHB development in a susceptible wheat cultivar.

266. Genetic and epigenetic changes in *Fusarium graminearum* following serial subculture. Rhaisa Crespo[2], Heather E. Hallen-Adams[1]. 1) Food Science and Technology, Univ of Nebraska-Lincoln, Lincoln, NE; 2) University of Puerto Rico, Mayaguez, Mayaguez, PR.

Fusarium isolates are notably unstable in culture and given to degradation unless certain precautions are taken. After only a few rounds of serial subculture, isolates can irreversibly lose the ability to form sporodochia, followed by conidia. The mechanism of these changes is unknown. To understand the nature of *Fusarium* morphological changes in culture, we began subjecting the sequenced strain of *F. graminearum* to serial subculture in July, 2011. Multiple lineages were begun from an initial soil stock, and each lineage is subcultured weekly, and a sample stored under glycerol at -80 C. For this study, we have performed shotgun pyrosequencing using 454 FLX-Plus on five of the lineages from one year after the study began; we have also sequenced one lineage from the beginning of the study (when all lineages should have been identical, and not significantly different from the published *F. graminearum* genome), and compared all to the published genome. Finally, we have used Illumina for bisulfite pyrosequencing to obtain methylation profiles.

267. Evolutionary and functional analysis of mitosis-related kinase genes in Fusarium graminearum. Huiquan Liu[1], Jiwen Ma[1], Shijie Zhang[1], Dawei Zheng[1], Juanyu Zhang[1], Chenfang Wang[1], Jin-Rong Xu[1,2]. 1) College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

Eukaryotic cell cycle is a series of recurrences of a defined set of events; during which, nuclear DNA is replicated in the S phase and segregated into two daughter nuclei during mitosis. To date, many protein kinases important for the onset and progression through mitosis have been identified. Most of these mitosis-related kinases are thought to be conserved from yeast to humans. In the model fungi used for cell cycle studies, including Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Aspergillus nidulans, the single copy cdc2 (CDC28) CDK gene is a key regulator of cell cycle essential for growth. However, the filamentous ascomycete Fusarium graminearum, the causal agent of wheat and barley head blight disease, has two putative Cdc2 orthologs. Either one of them is essential but deletion of both may be lethal. Whereas the cdc2B mutant has only minor defects in germination and growth, deletion of cdc2A had no obvious defects in growth but resulted in significant reductions in virulence. Ascosporogenesis but not perithecium formation or ascogenous hyphal growth was blocked in the cdc2A mutant. Mutants deleted of the single CDK kinase gene FgCAK1 had similar phenotypes with the cdc2A mutant. Both cdc2A and cdc2B interacted with itself and each other, and with FgCAK1. Therefore, cdc2A and cdc2B must have independent and overlapping functions in F. graminearum. It is likely that cell cycle regulation involves different cdc2 kinases and CDK activation mechanisms between vegetative and in plant growth. Infectious growth and ascosporogenesis may require only cdc2A, which is activated by FgCAK1. In addition, we found that F. graminearum has two Aurora protein kinase genes that are orthologous to yeast IPL1. The two F. graminearum Aurora kinases differ at the amino acid residue that is known to be related to different functions of Aurora A and Aurora B in humans, further indicating that yeast and F. graminearum differ in some key protein kinases involved mitosis. In addition, we systematically identified orthologs of other mitosis-related kinase genes in representative fungi. Although most of them are conserved across fungal tree of life, some mitosis-related kinase genes are lost or duplicated in certain lineages.

268. Functional analysis of A MADS-box transcription Mcm1 in *Fusarium graminearum* . Cui Yang[1], Guotian Li[2], Qian Zheng[1], Meigang Liu[1], Jin-Rong Xu[1,2], Chen fang Wang[1]. 1) NWAFU-PU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

Fusarium head blight, an important disease of wheat and barley is primarily caused by *Fusarium graminearum* in many parts of the world. In this homothallic ascomycete, sexual reproduction and the mating type locus play a critical role in its infection cycle because ascospores are the primary inoculum. In this study we identified and characterized the *FgMCM1* gene in *F. graminearum* that is orthologous to yeast *MCM1* MADS-box transcription factor. Deletion of *FgMCM1* resulted in the loss of perithecium production and pathogenicity. The *Fgmcm1* mutant rarely produced conidia with abnormal morphology and germination and was defective in response to various stresses. The FgMcm1-GFP fusion proteins localized to the nucleus and fully complemented the *Fgmcm1* mutant. Interestingly, approximately half of the sub-cultures of the *Fgmcm1* mutant often were significantly reduced in growth rate. These spontaneously occurred stunted subcultures had similar or more severe defects than the original *Fgmcm1* mutant in most of the phenotypes. In yeast two-hybrid assays, FgMcm1 interacted with Mat1-1-1, Fst12, and Tup1 but not with Mat1-2-1. The *Fgmcm1 mat1-1-1* double mutant was stable, suggesting that defects of the *Fgmcm1* mutant may be related to the interaction of *FgMCM1* with the other MAT TF genes. The *Fvmcm1* mutants of *F. verticillioides* had similar defects but were not unstable. To further understand the instability of the *Fgmcm1* mutant and slow growth subcultures, RNA samples isolated from the wild type, original *Fgmcm1* mutant, and a stunted subculture were sequenced. RNA-seq data and analyses will be presented. Overall, our data indicate that FgMcm1 may interact with MAT locus and other transcription factor genes to regulate cell identity and fungal development and pathogenesis in *F. graminearum*.

269. Genome sequencing of the *Fusarium graminearum* species complex in Korea. Haeyoung Jeong[1], Ulrich Güldener[2], Hee-Kyoung Kim[3], Seunghoon Lee[3], Theresa Lee[4], Sung-Hwan Yun[3]. 1) Systems & Synthetic Biology Research Center, KRIBB, Daejeon 305-806, South Korea; 2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München - German Research Center for Environmental Health, Ingolstädter Landstrabe 1, 85764 Neuherberg, Germany; 3) Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam 336-745, South Korea; 4) Microbial Safety Team, National Academy of Agricultural Science, RDA, Suwon 441-707, South Korea.

The *Fusarium graminearum* (*Fg*) species complex, the causal agent of Fusarium head blight of small grain cereals, comprises at least 15 lineages, or phylogenetically distinct species. Among these, lineages 6 (*F. asiaticum*) and 7 (*F. graminearum sensu stricto*) are major populations of the *Fg* complex recovered from rice and corn, respectively in Korea; lineages 3 (*F. boothii*) and 2 (*F. meridonale*) were also recovered from corn. The *F. asiaticum* population is clearly different from the *F. graminearum* population in terms of self-fertility, trichothecenes production, and host preference. We have sequenced the genomes of 19 Fg complex isolates belonging to the 4 clades or species found in Korea using 454 pyrosequencing or Illumina Hiseq technologies. As a representative genome sequence for *F. asiaticum*, we reconstructed five linear replicons from the *F. asiaticum* SCKO4 strain, which consist of four chromosomes, each corresponding to those of the previously sequenced *F. graminearum* strain PH-1 along with a separated small segment (451kb). By integrating multiple gene models that included the results obtained by *ab initio* gene prediction tools which incorporated RNA-seq data we manually identified a set of 12,448 protein-coding genes in SCKO4. Genome-wise comparison between SCK01 and PH-1 revealed a remarkable level of genomic synteny throughout the four chromosomes, but several rearrangements including inversions being located on chromosomes II and III in SCK04. Interestingly, the 451-kb fragment in SCK01 showed little sequence relatedness with the PH-1 genome. Similarly, we were able to assemble into 7 large contigs from the genome of a representative strain (GWS2-6-3) of *F. boothii*. Using these three representative genomes, we have intensively analyzed and compared the genomes of the *Fg* complex field isolates to provide insights into understating of evolutionary relationship among the *Fg* complex in Korea.

270. Identification and functional analysis of virulence genes in different host-pathogenic forms of *Fusarium oxysporum*. P. van Dam, S.M. Schmidt, M. Rep. Molecular Phyopathology, University of Amsterdam - SILS, Amsterdam, the Netherlands.

The species complex *Fusarium oxysporum* (*Fo*) represents one of the most abundant and widespread microbes of the soil microflora, including plant-pathogenic strains that, together, are able to infect a broad host range. In the tomato-pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), 11 small Secreted In Xylem (*SIX*) effector proteins were identified. These were later shown to be encoded on *Fol*'s mobile 'pathogenicity-chromosome' that can be transferred horizontally to non-pathogenic *Fo* strains, resulting in acquired pathogenicity. The goal of this project is to identify host-specific virulence genes in other *formae speciales* of *Fo*.

A representative set of isolates from different *formae speciales*, vegetative-compatibility groups and races will be selected for genome sequencing. The development of a comparative genomics bioinformatics pipeline, relying on sequence homology and specific patterns in the promoter regions (e.g. transposable elements) for identification of putative effector genes in newly sequenced *F. oxysporum* genomes takes a central position in our strategy. Using this approach, several putative effector genes were already identified in a strain of *Fo* f. sp. *melonis*, which infects muskmelon.

In combination with functional analysis of candidate effector genes, we want to compare the sets of virulence genes of *formae speciales* that infect members of the *Cucurbitaceae* and *Solanaceae* families, such as cucumber, muskmelon, watermelon, tobacco, sweet potato and eggplant. Newly discovered virulence genes can be used as molecular markers for diagnostic purposes. Additionally, phylogenetic analysis of virulence genes across *formae speciales* will help to reconstruct the evolution of host-specific pathogenicity in *Fo*, and the dynamics of the mobile accessory genome.

271. Protocol for generating gene knock-out transformants of the fungal pathogen Verticillium albo-atrum. M. Flajsman, S. Mandelc, B. Javornik. Univ of Ljubljana, Ljubljana, Slovenia.

A protocol for generating knock-outs of Verticillium albo-atrum, which is a destructive soilborne fungal pathogen that causes vascular wilt diseases, was successfully established. V. albo-atrum, along with V. Dahliae, is a significant source of crop plant disease, since between them they infect a broad spectrum of host species, from ornamental trees to major crops such as potato, tomato, cotton, tobacco and hop. The genom of V. albo-atrum has already been sequenced. Translation of genome sequence information into biological functions is therefore possible. One of the most powerful approaches for dissecting the gene function in phytopathogens is the study of the phenotypes of mutants in which a genomic locus has been altered by insertion (gene disruption) or replacement (gene replacement) with heterologous DNA. This is a high throughput reverse genetics approach, which greatly contributes to understanding the gene function of fungal pathogens. Our protocol for generating knock-outs of the fungal pathogen V. albo-atrum comprises two methods: the creation of knock-out plasmids by the USER Friendly cloning technique and transformation of the fungal pathogen by Agrobacterium tumefaciens-mediated transformation (ATMT). Knock-out strains of V. albo-atrum were made by site directed modifications of the pathogen genome by means of homologous recombination and achieved by introducing a DNA fragment containing two homologous recombination sequences flanking a selection marker. pRF-HU2 plasmid, containing a hygromycin resistance gene, was used for USER Friendly cloning of knock-out plasmids. Two PCR amplicons, containing homologous recombination sequences flanking a deletion gene, were inserted into the vector, which was used to transform E. coli cells and isolated plasmids were electroporated into A. tumefaciens. V. albo-atrum knock-outs were generated by ATMT. Knock-out strains for two genes found to be highly expressed at the protein level in the xylem of infected hop plants, were generated. V. albo-atrum knock-out transformants were verified by PCR testing and Southern blot analysis, which confirmed that deletion of the target gene had been successful. This is the first report, to our knowledge, of the creation of V. albo-atrum gene knock-outs. It demonstrates that knock-out transformants of this fungal pathogen can be efficiently made.

272. Functional analysis of catalase-peroxidase encoding genes in the fungal wheat pathogen *Zymoseptoria tritici*. A. Mirzadi Gohari[1,3], R. Mehrabi[1,2], P. J.G.M. de Wit[2], G. H.J. Kema[1]. 1) Wageningen University and Research Centre, Plant Research International, P.O. Box, 16, 6700 AA, Wageningen, The Netherlands; 2) Wageningen University, Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands; 3) Department of Plant Protection, College of Agriculture, University of Tehran, Plant Pathology Building, Karaj, Iran.

Zymoseptoria tritici is the new name of the foliar wheat pathogen cereal *Mycosphaerella graminicola* (teleomorph)/*Septoria tritici* (anamorph) that causes septoria tritici blotch particularly in regions with high rainfall and moderate temperatures during the production season. Similar to many plant pathogens, *Z. tritici* possesses three catalase-peroxidase genes that are known to detoxify H2O2 accumulated in the foliage during colonization. In the current study, we functionally analysed these three catalase-peroxidase genes and found that *MgCatD-1*, encoding a secreted catalase-peroxidase, plays an important role in the pathogenicity of *Z. tritici*. *MgDCat-1* mutants hardly induced any disease symptoms and expression analysis of *MgDCat-1 in planta* revealed that it is up-regulated during pathogenesis, particularly at 8 dpi (days post inoculation). This coincides with an important switch from a biotrophic to a necrotrophic lifestyle during pathogenesis suggesting that this gene is likely required to overcome H2O2-dependant defence responses during colonization. Furthermore, the *MgDCat-1* strain is hypersensitive to H2O2 as the spore germination dropped to 50% at 4mM H2O2 and to complete inhibition at 6 mM H2O2 compared to the WT IPO323 strain. These results show that secreted catalase-peroxidase is an important pathogenicity factor for successful pathogenesis of *Z. tritici*.

273. Efficient recycling of selective marker genes with the Cre-loxP recombination system via anastomosis in Cryphonectria parasitica. Dong-Xiu Zhang, Donald Nuss. Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD.

Reverse genetic analysis has played a significant role in advancing fungal biology, but is also limited by the number of available selectable marker genes (SMGs). The Cre-loxP recombination system has been adapted for use in filamentous fungi to overcome this limitation. Expression of the Cre recombinase results in excision of an integrated SMG that is flanked by loxP sites allowing subsequent rounds of transformation with the same SMG. However, current protocols for regulated expression or presentation of Cre require multiple time consuming steps. During efforts to disrupt four independent RNA-dependent RNA polymerase genes in a single strain of the chestnut blight fungus Cryphonectria parasitica, we tested whether Cre could successfully excise loxP-flanked SMGs when provided in trans via anastomosis. Stable Cre-producing donor strains were constructed by transformation of wild-type C. parasitica strain EP155 with the Cre coding domain under the control of a constitutive promoter. Excision of multiple loxP-flanked SMGs was efficiently achieved by simply pairing the Cre donor strain and the loxP-flanked SMGs transformed recipient strain and recovering mycelia from the recipient colony just above the anastomosis zone. This method was shown to be as efficient as and much less time consuming than excision by transformation-mediated expression of Cre and should be applicable for optimizing reverse genetics analysis in a broad range of filamentous fungi.

274. RNA-seq reveals the pleiotropic regulating functions of the transcription factor XYR1 in *Trichoderma reesei*. Liang Ma[1], Lei Zhang[1], Ling Chen[1], Gen Zou[1], Chengshu Wang[2], Zhihua Zhou[1]. 1) Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 2) Key Laboratory of Insect Developmental and Evolutionary Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

Trichoderma reesei is a well-known cellulase producer and widely applied in enzyme industry. XYR1 has been demonstrated to be the main transcription activator of cellulase and hemicellulase gene expression in *T. reesei*. To comprehensively investigate the genes regulated by XYR1, RNA-seq was used to determine the transcriptional profile of *T. reesei* Rut-C30 and the D*xyr1* strain cultured on either cellulose or glucose. A total of 467 distinct genes were classified as differentially expressed comparing parent strain with the *xyr1*-deleted strain when induced by cellulose, among which were mainly those involved in carbohydrate metabolism, lipid metabolism, protein fate and solute transport. Almost all the functional cellulase genes were found to be downregulated in the D*xyr1* strain while all the differentially expressed protease genes were upregulated. Furthermore, the expression of 84 genes identified as cellulose-induced were significantly impaired when *xyr1* was deleted. In contrast, 281 genes showed expression difference in the D*xyr1* strain when cultured on glucose as carbon source, and most of these were involved in solute transport, lipid metabolism, secondary metabolism and amino acid metabolism. In addition, we also found that the functional restoration of XYR1 in the D*xyr1* strain required the homologous insertion of *xyr1* at its original locus. Our study provides the global insight into the genes regulated by XYR1 beyond the hydrolase-encoding genes in *T. reesei* and might aid future studies to improve its cellulase production.

275. Tyrosinase an important enzyme for melanin production in the oomycete Saprolegnia parasitica. Marcia Saraiva[1*], Irene de Bruijn[2], Laura Grenville-Briggs[3], Debra McLaggan[1], Vincent Bulone[3], Pieter van West[1]. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; 2) Laboratory of Phytopathology, Wageningen University, 6709 PD Wageningen, Netherlands; 3) KTH - Royal Institute of Technology, School of Biotechnology AlbaNova University Center SE-106 91 Stockholm Sweden.

Filamentous fungi posses a pigment that often is considered an indirect virulence factor giving the fungi leverage. The pigment, melanin, acts as a protective agent against several threats and hazards that these organisms may encounter such as oxidants, killing by macrophages, UV and also antimicrobial compounds. In the biosynthetic pathway of melanin Tyrosinase is the first enzyme involved. Tyrosinases are widely distributed in nature, where they have been found in prokaryotes, eukaryotic microorganisms, invertebrates, plants and mammals. Here we describe the first functional characterisation of a tyrosinase (SpTyr) from the fish pathogenic oomycete Saprolegnia parasitica. This aquatic water mould infect a wide range of fish, amphibians and crustaceans that are relevant to the aquaculture industry and aquatic ecosystems, causing a disease called Saprolegniosis. This disease is responsible for millions of losses in aquacultures. Previously we found that SpTyr is highly expressed in sporulating mycelium. After developing and implementing a transient gene silencing method (RNAi), based on the delivery of in vitro synthesized dsRNA into protoplasts of this water mould, we obtained SpTyr-silenced lines with a significantly decreased tyrosinase activity of 40-60% compared to control lines. The tyrosinase activity correlated directly with the level of SpTyr-silencing in the transient lines, which ranged from 68.7-37.5%. Furthermore, the melanin content was measured spectrophotometrically in the SpTyr-silenced lines and found to be significantly reduced to 2-70%. Microscopic observations of SpTyr-silenced lines resulted in aberrant zoosporangium formation, with less pigment and abnormal morphology. Moreover, our results demonstrate that transient gene silencing can be successfully used to functionally characterise genes in S. parasitica and can provide a high-throughput tool for S. parasitica functional genomics.

276. Comparative transcriptomics of *Cordyceps bassiana* to understand expression levels of its NRPS related genes. B. Shrestha[1], J. Oh[2], M.-W. Hyun[3], J.-G. Han[1], H.-W. Kwon[3], S.-H. Hyun[2], S. H. Kim[3], H.-K. Choi[2], G.-H. Sung[1]. 1) Mushroom Research Division, Rural Development Administration, Suwon 441-707, Republic of Korea; 2) College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea; 3) Department of Microbiology and Institute of Basic

Sciences, Dankook University, Cheonan 330-714, Republic of Korea.

Cordyceps bassiana (= *Beauveria bassiana*) is a widely distributed entomopathogenic fungus, particularly important in the industry of biological control agents against agricultural pests and considered as a model organism for understanding fungal entomopathogenicity. We conducted a genome data mining of *C. bassiana* C101 isolate for nonribosomal peptide synthetase (NRPS) and PKS-NRPS hybrid gene clusters. To identify NRPS related gene clusters, whole genome sequence of *C. bassiana* C101 was subjected to antiSMASH program with default settings. Their domain sequences were predicted based on fungal-specific HMMER models using databases of Pfam and InterPro. A domain specificity signatures were derived by NRPSpredictor2 while substrate specificity prediction of the acyltransferase (AT) domains was based on web server SBSPKS. We identified 16 NRPS, 5 NRPS-like and 3 PKS-NRPS hybrid gene clusters, of which three are known for their metabolites (i. g., beauvericin, bassianolide and tenellin). For transcriptome analysis of NRPS related genes using Illumina RNA-Seq, *C. bassiana* C101 isolate was inoculated on SDAY, iron-, nitrogen- and lipid-rich media at 25°C and harvested to obtain its mycelia after two weeks of incubation. The stromata and perithecia of *C. bassiana* were also cultivated on brown rice medium and harvested from 4 weeks to 8 weeks old cultures at weekly intervals. As a result, beauvericin synthetase, a NRPS synthesizing insecticidal compound beauvericin, was highly expressed at mycelium grown in dark on SDAY but not in stromata except very low expression at 8 weeks old stromata. Conversely, another NRPS bassianolide synthetase, synthesizing insecticidal compound bassianolide, was not detected under any condition except very low detection in 8 weeks old stromata. Tenellin synthetase, a PKS-NRPS hybrid, was expressed only in stromata. Some NRPSs showed expression in both mycelia and stromata while others were expressed either in mycelia or stromata. In particular, the expression levels of beauvericin synthetase are correlated with the metabolic profiling of beauvericin in mycelia and stromatal conditions, indicating that the detailed information of the expression levels of NRPSs can be used to discover the metabolic diversity of *C. bassiana* with the further study of gene function and comparative metabolic profiling.

277. Global analysis of the *Colletotrichum gloeosporioides* genome and transcriptome reveals a conserved role for pacC pH regulation in fungi. Noam Alkan[1], Xiangchun Meng[3], Eli Reuveni[1], Gilgi Friedlander[1], Serenella Sukno[4], Michael Thon[4], Robert Fluhr[1], Dov Prusky[2]. 1) Plant Sciences, Weizmann Institute of Science, Rehovot, Israel; 2) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel; 3) Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China; 4) Department of Microbiology and Genetics, University of Salamanca, Villamayor, Spain.

Colletotrichum gloeosporioides, a widely distributed economically important agent in postharvest fruit disease, thrives by massive secretion of ammonia. The alkalized environment hijacks host response and activates fungal pathogenicity genes. In contrast, other types of fungi acidify the environment for their optimal growth. In both cases the pacC transcription factor, with homologs in yeasts and fungi, exerts critical pH control of gene expression. To explore global aspects of pH-dependent gene expression, we sequenced the *C. gloeosporioides* genome and compared transcriptomes of WT and a *DpacC* mutant to examine pacC regulation. The 54 Mb *C. gloeosporioides* genome comprises 16,603 transcripts. Transcriptome analysis of the mutant showed that *DpacC* regulates more than 5% of the fungal genome including; transporters to maintain cellular homeostasis, cell wall degrading enzymes to optimize pathogenicity and GATA-like transcription factors. The predictions were verified by monitoring gene expression in different media and during fruit infection a well as by pathogenicity assessment of selected deletion mutants. Analyses showed over-representation of pacC binding sites in the promoters of the pacC up-regulated genes. However, in the promoters of the down-regulated genes GATA-like binding sites were dominant. The results suggest duality in global pacC control; direct regulation of alkaline-induced transcripts but indirect regulation, by activation of other transcription factors to down-regulate acid expressed transcripts. To establish the generality of this scenario, conservation of pacC distribution was examined in genes selected by homology from 5 different fungi that have contrasting alkaline or acidifying pathogenicity strategies. The results showed that irrespective of pathogen colonization strategy, the homologs of up-regulated genes had over-representation of pacC binding sites. Significantly, the homologs of down regulated genes revealed cross-genome over-representation of GATA transcription factor binding sites. Thus, regulation by pacC is a phylogenetically conserved fungal mechanism exerting dual pH control for maintaining homeostasis and pathogenicity in changing environments.

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279. *Anisogramma anomala*: a unique fungus with a huge genome. Guohong Cai, Thomas Molnar, Bradley Hillman. Plant Biology and Pathology, Rutgers The State University of New Jersey, New Brunswick, NJ.

Anisogramma anomala (*Ascomycota*: *Diaporthales*) is the causal agent of hazelnut eastern filbert blight (EFB). This obligate and biotrophic fungus only reproduces sexually through ascospore. EFB is the most important disease of commercially grown hazelnut (European hazelnut), *Corylus avellana*, in the United States. It only causes minor disease on its natural host, the American hazel *C. Americana*, which is an understory shrub commonly found in deciduous forests in Northeast region of North America. Despite quarantine efforts, EFB was found in Washington in the late 1960s and reached Oregon's Willamette Valley, the main hazelnut production area, in 1986. Commercial cultivars carrying a single locus from cultivar Gasaway confer complete resistance to EFB in the west coast. However, certain strains from the east can break the resistance. We sequenced an Oregon strain using Illumina GA IIX platform. Approximately 26M 146 bp paired-end reads were used to produce a draft assembly with contig N_{50} of 10,384 bp and scaffold N_{50} of 32,987 bp. Excluding gaps, total assembly was 333.6 Mb. This is huge for a fungus with single nucleus. The genome size produced by draft assembly was corroborated by flowcytometry, which measured the genome size at approximately 370 Mb. The genome has low GC ratio of 32%. Approximately 85% of assembled genome is repetitive, and AT-rich sequences are enriched in the repetitive regions. We are exploring the genome sequences looking for answers to the uniqueness of this fungus.

280. *Leptosphaeria maculans* 'brassicae': "Transposable Elements changed my life, I feel different now". Jonathan Grandaubert[1], Conrad Schoch[2], Hossein Borhan[3], Barbara Howlett[4], Thierry Rouxel[1]. 1) INRA-BIOGER, Thiverval-Grignon, France; 2) NCBI, National Institutes of Health, Bethesda, MD, USA; 3) AAF Saskatoon, Canada; 4) School of Botany, University of Melbourne, Australia.

The Dothideomycetes phytopathogens *Leptosphaeria maculans* and *Leptosphaeria biglobosa* form a complex of 8 species and putative subspecies suggested to have diverged "recently". In 2007, the sequencing of an isolate of *Leptosphaeria maculans* 'brassicae' (Lmb) provided the first reference genome for this fungus. The 45-Mb genome has an unusual bipartite structure, alternating large GC-equilibrated and AT-rich regions. These AT-rich regions comprise one third of the genome and are mainly composed of mosaics of truncated Transposable Elements (TEs) postulated to have "invaded" the genome 5-10 MYA; they also comprise 5% of the predicted genes of which 20% encode putative effectors. In these regions, both genes and TEs are affected by Repeat Induced Point mutation (RIP). To investigate when and how genome expansion took place in the evolutionary series, and the consequences it had on fungal adaptability and pathogenicity, the genomes of five members of the species complex showing contrasted host range and infection abilities were sequenced. In silico comparison of the reference genome with that of 30-32-Mb genome of *L. maculans* 'lepidii' (Lml), *L. biglobosa* 'brassicae', *L. biglobosa* 'thlaspii' and *L. biglobosa* 'canadensis', showed these species have a much more compact genome with a very low amount of TEs

(<4%). The TE annotation allowed us to identify 121 TE families, all RIP-affected including the expected presence of lineage-specific TEs. Unexpectedly, two of the most expanded TE families in Lmb have been present in the Dothideomycete lineage for 100 million years. This questions how these families, while they have been anciently RIPped, managed to expand recently in Lmb. Interestingly, the comparison between the TE-rich genome of Lmb and the TE-poor genome of Lml, estimated to have diverged 5.5 MYA, indicated a nearly perfect synteny at the chomosomal level, suggesting low incidence of TE expansion on genome reorganisation. The gene annotation produced a similar gene number in each genome (~11000), but compared to the reference genome, less than 20% of the effector genes and 50% of other genes in AT-rich regions are present in the other genomes, suggesting that TEs were key players in gene innovation and that the genome environment promoted rapid sequence diversification and selection of genes involved in pathogenicity.

281. Genomics of fungal interactions for bioenergy crops. Igor Grigoriev. Fungal Genomics Program, US DOE Joint Genome Institute, Walnut Creek, CA.
Bioenergy crops depend on plant interactions with fungi including pathogens and symbionts. Saprobic fungi determine efficiency of plant material bioconvertion. Genomics offer keys to better understand molecular mechanisms of these interactions. The US Department of Energy Joint Genome Institute in collaboration with its user community leads massive genome sequencing and exploration of diverse fungi. Genome and transcriptome data produced for over 200 fungal species along with comparative genomics tools enable us to compare attributes of different lifestyle on genomic level. Comparison of large and diverse groups of fungi with similar lifestyle lead to genomic characteristics of their common traits. Interestingly, plant parasites, symbionts and saprobes reveal similar features encoded in their genomes, variations of which may correlate with their lifestyle. At the same time, expression, regulation, and evolution of these features ultimately determine the lifestyle of a fungus and require functional genomics efforts of the scale similar to that of modern genome sequencing.

282. Genomic analysis of oleaginous fungi. H. Koike[1], I. Takeda[1], T. Ishii[2], M. Umemura[2], K. Tamano[2], S. E. Baker[3], M. Machida[2]. 1) Bioprocess Research Institute, AIST, Tsukuba, Japan; 2) Bioprocess Research Institute, AIST, Sapporo, Japan; 3) Pacific Northwest National Laboratory, WA.
Microbial production of fats and oils is being developed as a means of converting biomass to biofuels. Some fungal species such as *Mortierella* and *Aspergillus* species are known to be oleaginous, accumulating fats and related compounds to more than 20% of their biomass. We have performed genomic analyses of oleaginous fungal species, intending future genetic engineering of *Asperigillus oryzae* as a host for the overproduction of fats and related compounds. We have sequenced and assembled draft genomes of three species (four strains) of lipid overproducing *Mucoromycotina* fungi using SOLiD 5500xl sequencer. The gene expression profiles under the lipid producing conditions have been analyzed by RNA-seq. We generated de novo assembly of the *Mucoromycotina* genomes using 50 bp reads generated from mate-paired libraries with 2.5 - 3.0-kb inserts. The assembled scaffolds of 4 species showed N50 values of 58 -477 kb and the maximum scaffold lengths of 247kb - 1.1 Mb. We have identified approximately 10,000 genes in the genome using ALN and GlimmerHMM. Comparative genomic analysis showed their phylogenetic relationships between each other and with other *Mucoromycotina* species whose genomes have been sequenced. The gene expression profiles of *Mucoromycotina* fungi were compared with each other and with those of *Aspergillus oryzae* to deduce induced and repressed metabolic pathways in terms of fats biosynthesis. We are intending to establish effective methods to over-produce fatty acid, secondary metabolites, and other related compounds useful for biofuel and/or chemical industry using *Aspergillus oryzae*.

283. Genome sequencing, assembly and annotation of a marine fungal isolate of Scopulariopsis brevicaulis using three different next generation sequencing technologies. Abhishek Kumar, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.
Several hundreds of marine fungi species are known which possess unique features, as they have to adopt to the marine environment. The enormous biodiversity of marine fungi is mirrored by the molecular diversity of their secondary metabolites. However, very little is known about marine fungal genetic resources, as they are ignored because they constitute very small fraction of over million of fungal species. Here, we report the first genomic sequence of a marine isolate of Scopulariopsis brevicaulis. S. brevicaulis is known to produce the cyclic peptides Scopulariade A and B. This species is common soil saprophyte and has been isolated from a wide variety of substrates. Some species of this genus are reported to cause human diseases. We have established the genomic sequence of a marine isolate of S. brevicaulis using three different next-generation sequencing methods namely, Roche 454, Illumina and Ion-torrent. The assembled genome of S. brevicaulis is ~32 Mb in size with N50 equals to 88 kb and 935 contigs containing 16298 genes with average intron length equals to 129.4. During annotation process, we were able to annotate 9340 genes (57.31 %) while 6958 genes (43.69 %) remained non-annotated in S. brevicaulis genome. This genome has 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene is hybrid NRPS-PKS.

284. Genome sequencing, assembly and annotation of a marine fungal isolate of Pestalotiopsis using next generation sequencing technologies. Abhishek Kumar, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.
Next-generation sequencing (NGS) has rapidly provided large wealth of applications of genomics to biology and medicine. We used NGS to sequence genome of Pestalotiopsis, a marine fungal isolate, known to produce wide variety of secondary metabolites with potentials in cancer treatment. We have established the genomic sequence of this marine isolate using two different next-generation sequencing methods namely, Roche 454 and Illumina HiSeq 2000. The assembled genome size of Pestalotiopsis is ~46 Mb with N50 equals to 71.9 kb and 4186 contigs containing 23492 genes, which is surprisingly very high for a fungus. The average intron length and the average intron per gene are 126.8 and 2.2, respectively. During annotation process, we annotated 60% genes of Pestalotiopsis genome with 44 NRPSs, 62 PKSs and 7 hybrid NRPS-PKS genes. Under EU-funded marine fungi project, these secondary metabolites are further characterized for their roles in cancer treatment.

285. Genome sequencing, assembly and annotation of a marine fungal isolate of Calcasporium using different next generation sequencing technologies. Abhishek Kumar, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.
Biology has more sharpen weapon in terms of application of genomics due to rapidly evolving next-generation sequencing (NGS), which made large-scale sequencing affords to regular laboratory practice. We used NGS to sequence genome of Calcasporium, a marine fungal isolate, known to produce wide variety of secondary metabolites with potentials in cancer treatment. We have established the genomic sequence of this marine isolate using two different next-generation sequencing methods namely, Roche 454 and Illumina HiSeq 2000. The assembled genome size of as Calcariosporium sp. is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing 15459 genes. The percentage GC% for this genome is 50.7%. The average intron length and the average intron per gene are 121 and 2.1, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated for Calcariosporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes. Under EU-funded marine fungi project, these secondary metabolites are further characterized for their roles in cancer treatment.

286. Genome sequencing, assembly and annotation of three marine fungal isolates using different next generation DNA sequencing methods for pharmaceutically important secondary metabolites. Abhishek Kumar, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.

Next-generation sequencing (NGS) techniques have changed the facets of genomics and its application. Until now marine fungal isolated are neglected in fungal genetics, largely due to fact that fungi have enormous diversity on land itself. We aimed to explore marine fungal isolates and their encoding natural products as drugs against cancer under the EU-funded project marine fungi (www.marinefungi.eu). We have taken three marine isolates namely, Scopulariopsis brevicaulis, Pestalotiopsis sp. and Calcarisporium sp. We have established the genomic sequences from these marine isolates of using three different next-generation sequencing methods (Roche 454, Illumina and ion-torrent) and predicted genes are presently in process of validation using illumina based RNA-seq. We are also comparing wild type phenotypes with higher -yielding mutants of these fungi with special interest on specific natural compound. The assembled genome of Scopulariopsis brevicaulis is ~32 Mb in size with N50 equals to 88 kb and 935 contigs containing 16298 genes with average intron length equals to 129.4. During annotation process, we were able to annotate 9340 genes (57.31 %) while 6958 genes (43.69 %) remained non-annotated in Scopulariopsis brevicaulis genome. This genome has 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene is hybrid NRPS-PKS. Similarly, the genome size for Pestalotiopsis sp. is~46 Mb with N50 equals to 71.9 kb and 4186 contigs containing 23492 genes, which is surprisingly very high for a fungus. The average intron length and the average intron per gene are 126.8 and 2.2, respectively. During annotation process, we annotated 60% genes of Pestalotiopsis genome with 44 NRPSs, 62 PKSs and 7 hybrid NRPS-PKS genes. The assembled genome size of as Calcariosporium sp. is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing 15459 genes. The percentage GC% for this genome is 50.7%. The average intron length and the average intron per gene are 121 and 2.1, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated for Calcariosporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes.

287. From Shiitake genomics to comparative mushroom genomics: Mushroomics. Hoi Shan Kwan, Chun Hang Au, Man Chun Wong, Jing Qin, Yung Yung Lee, Kin Sing Wong, Lei Li, Qianli Huang, Wenyan Nong, Man Kit Cheung, Jinhui Chang, Xuanjin Cheng. School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China.

The era of mushroom "omics" has come amongst the mushrooming of "omics" in all life science fields. I propose to name “omics” of mushrooms "Mushroomics". My laboratory has been working on the genomics and transcriptomics of the wood-degrading fungus *Lentinula edodes*, Shiitake mushroom, one of the most important cultivated mushrooms. We sequenced the genome of the monokaryon L54A using Roche 454 and ABI SOLiD sequencing platforms. Over 13,000 protein-coding genes were predicted. We constructed a high-density genetic linkage map that was useful to link scaffolds into super-scaffolds. The *L. edodes* genome assembly revealed a genome size of about 40 Mb. We performed RNA-Seq of multiple stages of *L. edodes*. For comparison, we also analyzed the transcription profiles of different stages of the model mushroom *Coprinopsis cinerea* using NimbleGen microarrays. Genes differentially expressed during fruiting body initiation and development in these mushrooms were identified. We conducted comparative analyses on publicly available genome sequences of basidiomycetes and ascomycetes, and revealed genes expanded in genomes of mushroom-forming fungi. The expanded genes included specific types of regulators, ubiquitin ligases, protein-binding proteins, protein kinases, and transcription factors. In particular, F-box and paracaspase domain proteins were significantly expanded. The cataloging of the unique composition of plant biomass-degrading enzymes in *L. edodes* genome revealed lignin-degrading laccases and manganese peroxidases, and multiple polysaccharide-degrading enzyme families, such as glycoside hydrolase families that target beta-glucans and pectin. We compiled the genome sequences of *L. edodes* and other fungi into an Ensembl-based platform, equipped with a battery of genomic analysis tools, for comparative mushroom genomic analysis. Our works have generated rich resources for the analysis of genomics and transcriptomics of mushroom-forming fungi. Our analysis also provided insights into the molecular mechanisms of fruiting body development in fungi and the evolution of fungal complex multicellularity. Indeed, our works showed that the era of "Mushroomics" has arrived.

288. Comparative genomics of *Ceratocystis polonica* and *Ophiostoma bicolor*, two bark beetle-associated pathogenic fungi. Ljerka Lah[1], Tom Hsiang[2], Colette Breuil[3], Joerg Bohlmann[4], Radovan Komel[1,5]. 1) Lab. for Mol. Biol, National Inst. of Chemistry, Ljubjana, Slovenia; 2) School of Environmental Sciences, University of Guelph, Guelph, Canada; 3) Dept. of Wood Science, Faculty of Forestry, University of British Columbia, Vancouver, Canada; 4) Michael Smith Laboratories, University of British Columbia, Vancouver, Canada; 5) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia.

Outbreaks of bark beetles and their pathogenic fungal associates are responsible for killing conifer trees and destroying forests worldwide. For example, in British Columbia, the mountain pine beetle and its major associated fungal pathogen *Grosmannia clavigera* have destroyed over 17 million hectares of pine forests. The sequenced genome of *G. clavigera* has provided insight into fungal mechanisms involved in resistance to conifer defense compounds, and into population genomics of this species. In Europe, where outbreaks of the European spruce bark beetle (*Ips typographus*) threaten Norway spruce (*Picea abies*), genomic resources are lacking for pathogenic fungal associates, *Ceratocystis polonica* and *Ophiostoma bicolor*. The aim of our work is to create these resources and to compare the genomes of these species with the annotated *G. clavigera* genome. The sizes of *C. polonica* and *O. bicolor* genomes, sequenced using Illumina and assembled using Abyss ver. 1.3.4, are 34.7 and 27 Mb, respectively. We identified (with Augustus ver. 2.6.1) and functionally annotated 6405 genes in *C. polonica* and 7746 in *O. bicolor*. We report results from preliminary analyses on gene family evolution, and on genes that code for enzymes important in eliminating and modifying conifer defense compounds, and those involved in specialized metabolism. The number of genes coding for ABC-transporters is approximately the same in all three species, while *G. clavigera* has more cytochrome P450 genes, polyketide synthases and ribosomal peptide synthases than either European bark beetle associate. Our results lead us to hypothesize that despite the fact that these species occupy apparently similar ecological niches, there are differences in their metabolism of host defense compounds and specialized metabolism.

289. The largest fungal mitochondrial genome of a basidiomycete contains signs of genetic flexibility and recombination events. Taina K. Lundell[1], Heikki Salavirta[1], Ilona Oksanen[1], Jaana Kuuskeri[1], Miia Mäkelä[1], Pia Laine[2], Lars Paulin[2]. 1) Microbiology and Biotechnology, Department of Food and Environmental Sciences, Viikki Campus, University of Helsinki, FI-00014 Helsinki, Finland; 2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, Viikki Campus, University of Helsinki, FI-00014 Helsinki, Finland.

Background and results. As the first part of de novo genome sequencing of the biotechnologically important, wood-decaying enzyme producing white-rot Basidiomycota species *Phlebia radiata*, we first assembled and gene annotated its mitochondrial genome (mtDNA) using 454 sequencing. The *P. radiata* mtDNA of 157 kb in size is the largest mitochondrial genome among fungi sequenced and characterized so far. The genome assembled as a single circular dsDNA molecule containing over 100 open reading frames. However, almost 80% of the mt genome is comprised of non-coding, intergenic and intronic sequence regions. Genes for mt SSU and LSU rRNA, 28 tRNAs, and fifteen genes encoding the conserved protein subunits in the mitochondrial

inner membrane complexes I, III, IV and V are identified. Additional protein-coding ORFs (sum 39) are predicted including a gene for ribosomal protein (*rps3*), a viral RNA-directed DNA polymerase (reverse transcriptase), and a gene for bacterial-originated DNA-directed DNA polymerase II of family B (*dpoB*). Total of 57 intron-homing endonucleases with core LAGLIDADGD and GYI-YIG domains were recognized in over 30 group I and II type introns, up to 3.4 kb in length, in ten of the fifteen conserved genes (*cox1,2,3; cob; nad1,2,4,4L,5; rnl*). Multigene phylogeny of the conserved proteins confirms current fungal taxonomy and a common, single origin of the mtDNA within Basidiomycota. **Conclusions.** The exceptionally large mt genome is explained by long intergenic stretches of DNA carrying repetitive and partially overlapping sequence elements, presence of additional open reading frames with unknown function, existence of the 6.1 kb duplication-inversion, and due to frequent intron splicing of the coding sequences. A few of the qualities indicate plasmid or viral origin, such as the *dpoB*, and the *cob* gene-interrupting long group II intron with reverse-transcriptase ORF. These features together with the duplicated inversion and dense repeat stretches, and the long introns with intron-associated homing endonucleases are indications of genetic flexibility, not previously recognized to such extent in fungal mitochondrial genomes. Thus, it may be concluded that DNA recombination as well as regulation of gene transcription are allowed and on-going events in the *P. radiata* mt genome.

290. *De Novo* Assembly of Fungal Genomes and Detection of Structural Variation using Extremely Long Single-Molecule Imaging. Nicholas R. Rhind[1], Alex Hastie[2], Ernest Lam[2], Andy Nguyen[2], Han Cao[2]. 1) Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA; 2) BioNano Genomics, San Diego CA.

De novo genome assemblies using only short read data are generally incomplete and highly fragmented due to the intractable complexity found in most genomes. This complexity, consisting mainly of large duplications and repetitive regions, hinders sequence assembly and subsequent comparative analyses. We have overcome these problems in four fungal genomes by assembling whole genome shotgun (WGS) contigs with a high resolution long range physical-mapping strategy. We used a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps assembled *de novo* from the extremely long single molecules retain the original context and architecture of the genome, making them extremely useful for structural variation and assembly applications.

We have built full genome assemblies of the four fission yeast genomes: *Schizosaccharomyces pombe*, *S. japonicus*, *S. octosporus* and *S. cryophilus*. The *S. pombe* assembly is similar to the published finished *S. pombe* genome, but reveals several complicated genomic features that were mis-assembled by a clone-based approached. In addition, we have resolved centromeric and telomeric repeats that had not been assembled by traditional approaches. The *S. japonicus* and *S. octosporus* assemblies identify a number of mis-assemblies in the published WGS genomes. The *S. cryophilus* genome is the first genome-scale assembly of what was a draft assembly of WGS contigs.

Our strategy combining genome map-based scaffolding with deep sequencing offers an integrated pipeline for whole genome *de novo* assembly solving many of the ambiguities inherent when using sequencing alone. Additionally, genome maps serve as a much-needed orthogonal validation method to WGS assemblies. As a result, genome maps improve contiguity and accuracy of whole genome assemblies, permitting a more comprehensive analysis of functional genome biology and structural variation.

291. Discovering host specificity candidate genes of *Sporisorium reilianum* by genotyping mixed-variety offspring. T. Wollenberg[1,2], J. Donner[2], J. Schirawski[1,2]. 1) Microbial Genetics, RWTH Aachen University, Aachen, Germany; 2) Molecular Biology of Plant-Microbe Interaction, Georg-August-University Göttingen, Göttingen, Germany.

The biotrophic plant pathogenic basidiomycete *Sporisorium reilianum* exists in two host-specific varieties, *S. reilianum f. sp. zeae* (SRZ) and *S. reilianum f. sp. reilianum* (SRS). SRS causes head smut of sorghum and leads to weak symptoms on maize, such as phyllody in the inflorescences. SRZ causes head smut of maize and leads to the formation of red phytoalexin-containing spots on inoculated sorghum leaves. Plant infection results after pairwise mating of compatible haploid cells that fuse to form an infective dikaryotic filament. The fungus persists in the host plant until flowering time, and in the inflorescences develops into diploid spores that germinate by meiotic division to haploid cells. Haploid cells of the two varieties are mating competent and are able to infect both maize and sorghum with a very low infection rate. To identify the genetic basis for the difference in host specificity, we analyzed virulent and non-virulent segregants of a mixed-variety (SRZ x SRS) infection both phenotypically and genotypically by a PCR-based approach. We identified twelve chromosomal regions that were associated to the phenotypic behavior of the strains. This shows that host adaptation is a multi-genic trait. To identify associated genes we genotypically analyze a larger set of strains by genome-wide SNP comparison after Illumina re-sequencing. Genome comparison of mixed-variety offspring is a powerful tool to discover candidate genes involved in host specificity.

292. Comparing comparative "omics" in *Coccidioides spp*. Emily A. Whiston, John W. Taylor. Plant & Microbial Biology, U.C. Berkeley, Berkeley, CA.

The mammalian pathogens *Coccidioides immitis* and *C. posadasii* are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, all of *Coccidioides*' closest known relatives are non-pathogenic. In this project, we are interested in genome changes between the *Coccidioides* lineage and its relatives, and how these changes compare to recently published comparative and population genomics, and transcriptomics studies in *Coccidioides*. *Coccidioides* and its closest sequenced relative, *Uncinocarpus reesii*, are estimated to have diverged 75-80 million years ago. Here, we have sequenced the genomes of four species more closely related to *Coccidioides* than *U. reesii*: *Byssoonygena ceratinophila, Chrysosporium queenslandicum, Amauroascus niger* and *A. mutatus*. For each of these four species, we prepared genomic DNA Illumina sequencing libraries; the resulting genome assemblies ranged from 23-34Mb, with N50 of 90kb-205kb. Predicted genes were confirmed by RNAseq; the total number of genes ranged from 8,179-9,184. We assessed individual gene gain/loss, and gene family expansion/contraction in *Coccioides* using these new genomes and other recently published genomes from the Onygenales order, including the yeast-forming dimorphic pathogens *Histoplasma* and *Paracoccidioides*, and the dermatophytes *Microsporum* and *Trichopyton*. We have compared these results to genes identified in recently published *Coccidioides* "omics" studies that show evidence of positive selection, introgression and/or differential expression.

293. The transcriptional response during cell-fusion incompatibility in Podospora anserina. Frédérique Bidart, Sven J. Saupe, Corinne Clavé. IBGC, CNRS, Bordeaux, France.

Heterokaryon incompatibility is a form of non-self recognition common in filamentous fungi that occurs when filaments of different isolates of the same species fuse. Compatibility is controlled by so-called het loci and fusion of strains of unlike het genotype triggers a complex incompatibility reaction that leads to the death of the fusion cell. Herein, we analyze the transcriptional changes during the incompatibility reaction in Podospora anserina. The incompatibility response was found to be associated with a massive transcriptional re-programming: 2248 genes were up-regulated by a factor 2 of more during incompatibility. In turn, 2463 genes were down-regulated. HET and NACHT domains previously found to be involved in the control of heterokaryon

incompatibility were highly enriched in the up-regulated gene set. In addition, incompatibility was characterized by an up-regulation of secondary metabolism clusters, toxins and effector-like proteins, proteolytic and other hydrolytic activities. Genes for ribosome synthesis and energy production were in contrast down regulated. There was a significant overlap between regulated genes during incompatibility in P. anserina and N. crassa indicating communality in the incompatibility responses in these two species. In P. anserina, the up regulated set was found to be enriched for proteins lacking orthologs in other species and chromosomal distribution of the up-regulated genes was uneven with up regulated genes enriched in genomic islands and certain chromosomes. Globally, this study shows that transcriptome changes occurring during cell fusion incompatibility in P. anserina are massive and pleiotropic and in several aspects related to host-pathogen interactions described in other fungal species.

294. Comparative Analysis of Putative Rhodopsins in Early Diverging Fungal Lineages. Steven Ahrendt[1], Edgar Medina[1,2,3], Jason Stajich[1]. 1) Plant Pathology & Microbiology, University of California, Riverside, Riverside, CA; 2) Departamento de Ciencias Biológicas, Universidad de Los Andes, Bogotá, Colombia; 3) University Program in Genetics & Genomics and Department of Biology, Duke University, Durham, NC.

Species belonging to the early diverging zoosporic fungal lineages (Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota) reproduce via motile uniflagellated spores. Previous work has shown that some of these zoosporic fungi are phototaxic [1]; however, light sensing in zoosporic fungi has not been fully explored. The opsins are a broad class of photosensitive, seven-transmembrane G-protein coupled receptor proteins. One sub-class of opsin, the type 2 Rhodopsins, has previously only been identified in metazoan lineages [2]. Here we describe the identification and structural/functional analyses of a putative type 2 rhodopsin in several species of recently sequenced zoosporic fungi: *Batrachochytrium dendrobatidis*, *Spizellomyces punctatus*, *Allomyces macrogynus*, *Rozella allomycis*, *Gonapodya prolifera*, and *Homolaphlyctis polyrhiza*. Computational modeling of the *B. dendrobatidis* and *S. punctatus* proteins indicates that they both adopt the seven-transmembrane helix conformation typical of GPCRs. Additional observed motifs are the so-called "ion lock" and conservation of the retinal binding pocket. The *B. dendrobatidis* protein sequence is notably lacking the conserved lysine residue, however this residue is present in the *S. punctatus* sequence. The number of identified Ga proteins is roughly consistent among the basal lineages, the Zygomycetes, and the Dikarya. Comparative genomics analyses of rhodopsin and flagellar genes in the basal lineages, Zygomycetes, and Dikarya show a correlation of flagellum and rhodopsin presence across the fungi, suggesting an evolutionary linkage between light-sensing and motility during the transition from aquatic to terrestrial lifestyles. [1] Saranak & Foster. Nature. 1997. [2] Spudich et al. Ann. Rev. Cell Dev. Biol. 2000.

295. Molecular Tools to Silence and Confirm Genes in Phytophthora Sojae. Felipe R. Arredondo[1], Brett M. Tyler[1], Shiv D. Kale[2]. 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Virginia Bioinformatics Institute, Virginia Tech. Blacksburg, VA.

Bioinformatics analysis is a powerful tool that decreases the number of possible leads to confirm the function of an interesting gene. These leads are only virtual and theoretical but still have to be proven correct and accurate. PEG/ Protoplast transformation and particle bombardment transient expression have been part of Phytophthora molecular research for many years and valuable tools in gene identification and confirmation of function. Possibly there are many hundreds of genes in Phytophthora species involved during the infection mechanism; the function of the majority of these virulence genes is largely unknown. Introducing genes into P. sojae via PEG/Protoplast transformation is a reliable tool that can confirm gene function by over-expression or silencing. Another reliable tool is particle bombardment with the PDS1000 to transiently express intact or modified genes into soybean leaves. In this poster I will introduce the mechanics of these powerful tools.

296. Functional Characterization of Transcription Factor Genes, *MoNIT4* and *MoLEU3*, in *Magnaporthe oryzae*. Jaehyuk Choi[1], Soyeon Yoo[2], Yong-Hwan Lee[1,2,3]. 1) Center for Fungal Pathogenesis; 2) Department of Agricultural Biotechnology; 3) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Rice blast disease caused by *Magnaporthe oryzae* is one of the most destructive threats to global rice production. Nitrogen metabolism has shown to play an important role in pathogenicity of this fungus. Here, we characterized two genes encoding putative transcription factors belonging to the $Zn(II)_2Cys_6$ family, *MoNIT4* and *MoLEU3*, homologs of nit-4 in *Neurospora crassa* and leu3p in *Saccharomyces cerevisiae*, respectively. The D*Monit4* mutant showed reduction in conidiation and mycelial growth under nitrogen starvation compared to the wild-type. Addition of ammonium restored the growth defects in the mutant. The expression of nitrate and nitrite reductase genes was significantly reduced in the mutants, suggesting that these genes are under control of MoNIT4. The D*Moleu3* mutant exhibits severe defects in conidiation and pathogenicity as well as mycelial growth under nitrogen starvation. Addition of leucine complemented the defects in conidiation and mycelial growth in the D*Moleu3* mutant. The decreased expression of 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, and NADP-specific glutamate dehydrogenase genes supports that MoLEU3 functions as a regulator for leucine biosynthesis and ammonia assimilation pathways. Taken together, these findings will help to understand the nitrogen metabolism network in *M. oryzae* and its role in the development of the rice blast disease.

297. Comparative genomic analysis of world-wide *Magnaporthe oryzae* isolate collection. Jaeyoung Choi[1], Gir-Won Lee[2], Sook-Young Park[3], Junhyun Jeon[1], Jaehyuk Choi[3], Ki-Tae Kim[1], Yong-Hwan Lee[1,3,4]. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; 2) Department of Bioinformatics and Life Science, Soongsil University, Seoul 156-743, Korea; 3) Center for Fungal Pathogenesis; 4) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Since the genome sequence of *Magnaporthe oryzae* 70-15 was released, functional and comparative genomic researches have been extensively carried out. However, characteristics of essential genes in *M. oryzae* are largely unknown. In this study, we sequenced 39 *M. oryzae* isolates collected from world-wide and analyzed to find the set of core genes. The average assembly size and the total number of predicted genes in the 39 *M. oryzae* genomes were 37Mb and 14,517 genes, respectively. We found that a total of 5,148 genes turned out to be conserved at the amino acid level (identity ³ 80%) among the 40 *M. oryzae* genomes including strain 70-15. To investigate a set of the core genes, we applied three different databases: Fungal Transcription Factor Database (FTFD; http://ftfd.snu.ac.kr/), Fungal Cytochrome P450 Database (FCPD; http://p450.riceblast.snu.ac.kr/) and Fungal Secretome Database (FSD; http://fsd.snu.ac.kr/). In addition, three in-house databases were used for the prediction of cell wall-degrading enzymes (CWDEs), peroxidases and laccases. As a result, we found a core set of genes in *M. oryzae*: 932 secreted proteins, 269 transcription factors, 33 cytochrome P450 genes, 18 CWDEs, 17 peroxidases and 5 laccases. Furthermore, we discovered an overlap of 38 genes between the above groups which have secretory potential. To archive and manage those newly sequenced genomes, we developed the *Magnaporthe* Atlas (http://www.magnaporthe.org/) as a web-based solution. Our core gene set of *M. oryzae* will facilitate discovery of lineage-specific innovations with implications in coevolution with specific host cultivars.

298. Functional characterization of two genes encoding putative $Zn(II)_2Cys_6$ transcription factors, *MoCOD1* and *MoCOD2* in *Magnaporthe oryzae*. Hyunjung Chung[1], Sook-Young Park[2], Jeahyuk Choi[2], Junhyun Jeon[1], Yong-Hwan Lee[1,2,3]. 1) Department of Agricultural Biotechnology; 2) Center for Fungal

Pathogenesis; 3) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Transcription factors (TFs) play pivotal roles in regulation of gene expression during cellular processes. The rice blast fungus, *Magnaporthe oryzae* undergoes a series of morphological changes during the infection. To elucidate the roles of TFs in development of rice blast disease, two $Zn(II)_2Cys_6$ TF genes, *MoCOD1* and *MoCOD2*, were characterized. Both D*Mocod1* and D*Mocod2* mutants showed defects in conidiation and pathogenicity. Reduced pathogenicity of the D*Mocod1* mutant was due to defects in invasive growth while the D*Mocod2* mutant exhibited no pathogenicity. Especially, restricted invasive growth and accumulation of dark brown granules around infection hyphae were frequently observed in the D*Mocod2* mutant. The granule accumulation is considered as a plant defense response. Genetic complementation with the wild type alleles restored the defects in conidiation and pathogenicity. Taken together, both *MoCOD1* and *MoCOD2* are responsible for conidia development and pathogenicity in the rice blast fungus. This is the first report of the involvement of $Zn(II)_2Cys_6$ TFs in pathogenesis of fungal plant pathogens.

299. Comparative proteomics of monoclonal-antibody enriched haustoria from three races of *Puccinia triticina*. Christof Rampitsch[1], Eva Beimcik[1], Aslihan Gunel[2], Guus Bakkeren[3], Tao Fan[1]. 1) Cereal Research Ctr, Agriculture & Agrifood Canada, Winnipeg, Canada; 2) Ahi Evran University, Department of Chemistry, Kirşehir, Turkey; 3) Pacific Agrifood Research Centre, Summerland BC, Canada.

Puccinia triticina (Ptr) causes leaf rust on wheat and is a problem in most areas where wheat is grown. The host-pathogen interaction follows the gene-for-gene model, where an interaction between host resistance (*R*) genes and pathogen avirulence (*avr*) genes determines whether the plant will remain healthy, or whether the pathogen can complete its life cycle and cause disease. *Ptr* is an obligate parasite which penetrates wheat leaves through stomata, colonizes the apoplastic space and forms haustoria inside host cells. Haustoria mediate nutrient up-take between the host and pathogen thus play a major role in pathology. Purification of milligram quantities of haustoria from *Ptr* to >95% homogeneity, determined visually by calcofluor white staining, has been made possible through the development of specific monoclonal antibodies. The haustoria proteome is of great interest, since it likely contains proteins with potential roles in pathogenesis. We have purified haustoria from races 1, 9 and 161 for a comparative proteome analysis, because complete genomic sequences exists for these. This is an essential requirement for homology-based matching of mass spectral data. Haustoria are surrounded by the plant plasma membrane, an extrahaustorial matrix and extrahaustorial membrane. As a result, they are recalcitrant tissues that resist analysis by conventional proteomics approaches. We have designed a strategy for obtaining the maximum yield of tryptic peptides from purified haustoria, suitable for LC-MS analysis. Preliminary results of the comparative proteome will be presented and discussed.

300. RNA-seq analyses of gene expression in the microsclerotia of *Verticillium dahliae*. Dechassa Duressa[1], Amy Anchieta[1], Donquan Chen[2], Anna Klimes[3,4,5], Katherine F. Dobinson[3,4], Maria Garcia-Pedrajas[6], Steven J. Klosterman[1]. 1) USDA-ARS, Salinas, CA; 2) Comprehensive Cancer Center & Division of Preventive Medicine, University of Alabama, Birmingham, AL; 3) Department of Biology, University of Western Ontario, London, ON, Canada; 4) Agriculture and Agri-Food Canada, London, ON, Canada; 5) Department of Physiological and Biological Science, Western New England University, Springfield, MA; 6) Estación Experimental La Mayora CSIC, Málaga, Spain.

Verticillium dahliae is a soilborne fungus that causes wilt disease in plants. Verticillium wilt is difficult to control because the pathogen is capable of persisting in the soil for 10 to15 years as melanized microsclerotia, rendering crop rotation strategies for the control of this disease ineffective. The microsclerotia of *V. dahliae* produce infectious hyphae that give rise to primary infections. As such, the processes of microsclerotia formation, maintenance, and germination are critically important in the disease cycle of *V. dahliae*. To shed additional light on the molecular processes involved in microsclerotia biogenesis and melanin synthesis in *V. dahliae*, three replicate RNA-seq libraries were prepared from 10 day-old microsclerotia (MS)-producing cultures of *V. dahliae* (ave=52.23 million reads), and those not producing microsclerotia (NoMS, ave=50.58 million reads), and analyzed for differential gene expression. The comparisons revealed up-regulation of MS library genes involved in melanogenesis, including tetrahydroxynaphthalene reductase (344-fold increase) and scytalone dehydratase (231-fold increase), and of additional genes located in a 48.8 kilobase melanin biosynthetic cluster. Numerous hypothetical protein-encoding genes were also identified as differentially expressed in the MS library. For confirmation of differential expression, selected genes identified by RNA-seq as up- or down-regulated were analyzed by RT-qPCR of RNA from several MS and NoMs culture types, including MS cultures that were stored for 6 months at 4°C, and seven day old cultures having an intermediate number of melanized MS. These data provide further insight into gene expression during melanin biosynthesis and MS formation in *V. dahliae*, and the products encoded by these genes may represent alternative disease control targets.

301. Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis. Francis M. Martin, Mycorrhizal Genome Initiative Consortium. Tree-Microbe Interactions, Lab of Excellence ARBRE, INRA-Nancy, Champenoux, France.

Genomics has introduced an important new dimension into mycorrhizal research by establishing data to serve as a new and fundamental resource for genetics and molecular biology of the symbiosis formation. With the current genomic view of ectomycorrhizal (EM) fungi that we have, a possible scenario suggests that (1) irreversible losses of lignocellulose decomposition pathways play a key role in the evolutionary stability of the ectomycorrhizal mutualisms and (2) that each major EM fungal clade has subsequently and independently designed symbiotic molecular toolboxes each time the mycorrhizal lifestyle has arisen in the tree of life. This hypothesis would predict that symbiotic toolboxes are tailor made for each major fungal clade (e.g., Agaricales, Boletales, Sebacinales) and may be tuned according to specific plant hosts. To further our understanding of the evolution of these symbioses, the Mycorrhizal Genomics Initiative targets a set of 30 fungal mycorrhizal species. Taxa have been selected for (1) their phylogenetic novelty, (2) their ability to establish different types of mycorrhizal symbioses and (3) their taxonomic relationships with already sequenced EM genomes. Several target species are capable of forming different types of intracellular colonizing structures - and this plasticity depends on plant host. Several species are dominant fungus in their ecological settings and others are currently used in the commercial forestry industry to inoculate conifer or hardwood seedlings for lumber, bioenergy and landscape trees. I will discuss how the comparative analysis of mycorrhizal genomes has, and will continue, to shed light on the evolution of mycorrhizal symbioses.

302. Known unknown genes: evolution of eukaryotic BEM46. Abhishek Kumar, Krisztina Kollath-Leib, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.

The bud emergence 46-like (BEM46) protein from Neurospora crassa belongs to the alpha/beta-hydrolase superfamily. Recently, we have reported that the BEM46 protein is localized in the perinuclear ER and also forms spots close by the plasma membrane. The protein appears to be required for cell type-specific polarity in Neurospora crassa. Furthermore, initial studies suggested that the BEM46 amino acid sequence is conserved in eukaryotes and is considered to be one of the widespread conserved “known unknown” eukaryotic genes. To unravel origin and molecular evolution of these genes in different eukaryotes, we carried out a comprehensive sequence, structural functional and phylogenetic analyses of BEM46 orthologs. During this study, we

found that all eukaryotes have at least a single copy of a bem46 ortholog. Upon scanning of these proteins from various spiecies, expansions leading into several paralogs in vertebrates and plants were identified. We illustrate insertion/deletions (indels) in the conserved domain of BEM46 protein, which allow differentiating fungal classes such as ascomycetes from basidiomycetes. Furthermore, we analyze several duplicates of this gene in different animal and plant genomes to understand possible mechanisms of evolution after separation from the fungal lineage. In addition, we unravel that BEM46 protein from N. crassa possess a novel endoplasmic-retention signal (PEKK) using GFP-fusion tagging experiments, hinting there is need to re-define the motifs in conserved in various protein sequences as over a million of genome sequences will be available in next decade.

303. Sugar 'cubed' - A Comparative Systems Analysis of Plant Cell Wall Polysaccharide Recognition and Degradation Using the Model Filamentous Fungus *Neurospora crassa*. J.P. Benz, S. Bauer, N.L. Glass, C.R. Somerville. Energy Biosciences Institute, UC Berkeley, Berkeley, CA.

Filamentous fungi are currently the primary source of plant cell wall degrading enzymes for the production of biofuels from lignocellulosic feedstocks. However, despite tremendous improvements of these enzyme cocktails over the last years, they are still rather inflexible and will not work optimally in situations such as complicated with a changing variety of feedstocks. Fungi have evolved with their host plants in a long and intricate relationship, and a detailed understanding of their responses to the various building blocks present in the plant cell wall material will also help to improve the industrial applicability and versatility of these enzyme cocktails. In recent years, the ascomycete *Neurospora crassa* has been developed as a model system to study cellulose and xylan degradation by filamentous fungi. As a complement to these studies, here we performed a systems analysis of pectin degradation, the third major plant cell wall polysaccharide. A combination of proteomics and transcriptomics was used to define the "toolbox" *N.crassa* uses to degrade this highly complex heteropolysaccharide, and to identify new components that seem to work both synergistically and antagonistically in this process. Moreover, in combination with the data from two earlier studies, describing the responses to cellulose and xylan, the acquired knowledge allowed for the first time to put the individual responses to each of these three main plant cell wall polysaccharides into perspective. Central to this analysis was the construction of a co-expression matrix covering the most relevant carbon source-related inducing conditions. The applicability of this matrix could be demonstrated by successfully guiding in the functional characterization of an unknown sugar transporter, which was identified to mediate L-arabinose uptake. Only if we understand the building blocks of the carbon-related response pathways we can attempt to put them together into the "bigger picture". The comparative approach presented here therefore is an important step towards a more profound understanding of the fungal degradation process of complex biomass.

304. Building upon whole genome resequencing in Neurospora. Kevin McCluskey, Aric Wiest, Robert Schnittker. Sch Biological Sci, Univ Missouri, Kansas City, Kansas City, MO.

The availability of whole genome sequence allows immediate comparison between polymorphisms that have physiological impact and those that are neutral. We are exploiting this as we characterize genes responsible for Acriflavine resistance. Preliminary analysis showed that despite a wealth of polymorphisms among whole genome sequenced strains, the ORF NCU09975 encoding an abc3 transporter is not altered in the acriflavine resistant strain FGSC 1215. Additional analyses have pointed to the transcription factor gene NCU09974 and the polymorphism in this gene in the acriflavine resistant strain is unique both in comparison to the reference genome strain and among the growing number of strains subject to whole sequencing. Continuing work aims to test whether transfer of the NCU09974 allele from the acriflavine resistant strain to an otherwise sensitive strain will confer resistance. Additional studies will investigate whether the broad resistance seen in classical acriflavine resistant mutants can allow identification of a compound that can be used as a selectable agent in combination with the newly identified allele.

305. Genome based phylogeny of early diverging fungal lineages. A. P. Gryganskyi[1], G. Bonito[1], M. Rodriguez-Carres[1], T. M. Porter[2], Y. Chen[3], S. Robb[4], H.-L. Liao[1], I. M. Anishchenko[5], O. V. Savytskyi[6], R. Ortega[1], J. E. Stajich[4], J. Heitman[3], A. P. Litvintseva[7], T. Y. James[8], S. Sekimoto[9], J. Spatafora[10], R. Vilgalys[1]. 1) Biology, Duke University, Durham, NC; 2) Ecology and Evolutionary Biology, McMaster University, Hamilton, ON, Canada; 3) Duke University Medical School, Durham, NC; 4) Plant Pathology and Microbiology, University of California, Riverside, CA; 5) Institute of Botany, NASU, Kyiv, Ukraine; 6) Institute of Molecular Biology and Genetics, NASU, Kyiv, Ukraine; 7) Centers for Disease Control and Prevention, Atlanta, GA; 8) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 9) Biological Sciences, The University of Alabama, Tuscaloosa, AL; 10) Botany and Plant Pathology, Oregon State University, Corvallis, OR.

The phylogeny of the early diverging fungal lineages remains controversial in spite of a growing database of morphological, ultrastructural, biochemical and molecular evidence. Here we present a comprehensive molecular phylogeny for the basal fungi using metagenomic data from 30 fungal taxa for which whole genomes or ESTs are available. Taxa include a dozen flagellated lineages, ten zygomycetous taxa, and key representatives of the Glomeromycota, Ascomycota, and Basidiomycota. Phylogenetic trees built from 434 orthologs (some missing data) and 29 orthologs (no missing data) are congruent and statistically well supported. Our results show a clear separation of most flagellated fungi from terrestrial taxa. An analysis of the presence of the genes associated with the flagellar apparatus supports the hypothesis that the flagellum was lost once concomitant with fungi transitioned to terrestrial habitats. Zygomycetous lineages occupy an intermediate position between flagellated fungi and the Dikarya with Entomophthoromycotina and Kickxellomycotina representatives as a basal clade.

306. Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum. Robert Riley[1], Asaf Salamov[1], Robert Otillar[1], Kirsten Fagnan[1], Bastien Boussau[3], Daren Brown[4], Bernard Henrissat[5], Anthony Levasseur[5], Benjamin Held[6], Laszlo Nagy[2], Dimitris Floudas[2], Emmanuelle Morin[7], Gerard Manning[8], Scott Baker[9], Robert Blanchette[6], Francis Martin[7], David Hibbett[2], Igor Grigoriev[1]. 1) Joint Genome Istitute, Lawrence Berkeley National Lab, Walnut Creek, CA; 2) Clark University, Worcester, MA; 3) UC Berkeley, Berkeley, CA; 4) USDA, Peoria, IL; 5) AFMB, Marseille, France; 6) UMN, St. Paul, MN; 7) INRA, France; 8) Salk Institute, La Jolla, CA; 9) Pacific Northwest National Lab, Richland, WA.

Fungi of the phylum Basidiomycota (basidiomycetes), make up some 37% of the described fungi, and are important in forestry, agriculture, medicine, and bioenergy. This diverse phylum includes symbionts, pathogens, and saprobes including wood decaying fungi. To better understand the diversity of this phylum we compared the genomes of 35 basidiomycete fungi including 6 newly sequenced genomes. The genomes of basidiomycetes span extremes of genome size, gene number, and repeat content. A phylogenetic tree of Basidiomycota was generated using the Phyldog software, which uses all available protein sequence data to simultaneously infer gene and species trees. Analysis of core genes reveals that some 48% of basidiomycete proteins are unique to the phylum with nearly half of those (22%) comprising proteins found in only one organism. Phylogenetic patterns of plant biomass-degrading genes suggest a continuum rather than a sharp dichotomy between the white rot and brown rot modes of wood decay among the members of Agaricomycotina subphylum. There is a correlation of the profile of certain gene families to nutritional mode in Agaricomycotina. Based on phylogenetically-informed PCA analysis of such profiles, we predict that that Botryobasidium botryosum and Jaapia argillacea have properties similar to white rot species, although neither has liginolytic class II fungal peroxidases. Furthermore, we find that both fungi exhibit wood decay with white rot-like characteristics in growth

assays. Analysis of the rate of discovery of proteins with no or few homologs suggests the high value of continued sequencing of basidiomycete fungi.

307. Functional analysis of roles of expanded genes in fruiting body development in Coprinopsis cinerea. Jinhui Chang, Hoi Shan Kwan. School of life sciences, The Chinese University of Hong Kong, Hong Kong.

We wish to study the relationship of expanded genes and evolutionary adaptation in mushroom-forming fungi. We planned to (1) detect the enrichment of expanded genes specific for mushroom-forming fungi, (2) develop a pipeline to construct protein-protein interaction (PPI) networks and match them with the established pathways in REACTOME database for fungal proteins, and (3) characterize the functions and action stage of the expanded kinases in fruiting body development. The expanded genes in late evolved organisms contribute to adaptive functions and morphological characters. Mushroom-forming fungi can be differentiated from the simple fungi by the extra morphological status of fruiting bodies. We hypothesize that the expanded genes unique to mushroom-forming fungi are critical for fruiting body development. By comparing 70 species from basidiomycota and ascomycota, we found significant enrichment in some protein functional clusters in mushroom-forming fungi comparing to simple fungi. Among these clusters, we chose to further analyze the group of Posttranslational modification related genes. We predicted and compared the PTM sites density. We found that the greater the genome size the lower the ubiquitylation site density . We developed a novel pipeline to search the literature for interacting proteins and construct PPI networks. With this pipeline, we proposed a light signal transduction phosphorylation cascade which involves some FunK1 kinases and components in PKA and MAPK pathways. To investigate the functional roles of these kinases in the putative cascade, we introduced the siRNA of corresponding genes into Coprinopsis cinerea at five stages in the life cycle. We showed by transient knock down of expanded kinases that they play imported roles in light signal transduction pathway and possess different functions in different developmental stages.

308. Comparative analysis of fungal kinomes. Yousef Shbat, Abhishek Kumar, Frank Kempken. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.

Many cellular processes are regulated by phosphorylation via protein kinases. To unravel the understanding of protein phosphorylation, normally the protein kinase complements (known as 'kinomes') are examined genome-wide in eukaryotic species. About 2% of eukaryotic genes are protein kinases. Bioinformatics and comparative genomics were used to determine kinomes from eukaryotes and to explore in evolutionary and functional context. Kinases are major regulators of cellular processes in fungi, in similar fashion as they regulate other eukaryotes. For example, 77 viable mutants for ser/thr kinase genes (of 86 in total) were identified in N. crassa and 57% illustrated at least one growth or developmental phenotype. Given that there is over 100 fungal genomes are known. Hence, there is a need of more comprehensive analysis of fungal kinomes. We have established kinomes of about 90 fungi with 5604 kinases, which also included pseudokinases (~1%). Fungi have expansion of ser/thr kinases in comparisons to other classes of kinases. We thus report how kinases in combination with ~80 other protein domains, evolved to perform their roles in different signalling cascades in these fungi. Furthermore, we annotated and analysed these kinases for evolutionary mechanisms operating in fungal kinomes.

309. VeA, VelB and FluG affect conidiation and aflatoxin production of Aspergillus flavus. P-K. Chang, L. Scharfenstein, P. Li, K. Ehrlich, B. Mack. Agricultural Research Service, Southern Regional Research Center, New Olreans, LA.

Asexual differentiation in Aspergillus nidulans involves complex control by a number of factors and is light-dependent. The VelB/VeA/LaeA complex in A. nidulans coordinates light signal with development and secondary metabolism. We investigated the roles of velvet family genes, veA, velB and velC, and fluG (fluffy phenotype in A. nidulans) in an aflatoxigenic Aspergillus flavus strain. Knockout strains of veA or velB conidiated poorly in the dark but not in the light. Knockout strains of fluG also showed decreased conidiation but had increased sclerotial production. Deletion of fluG in the veA or velB knockout resulted in a marked decrease in conidiation even in the light. Growth under stress (0.6 M potassium chloride) partially restored aforementioned defects in conidiation. The veA or velB knockout mutant but not the velC or fluG mutant was unable to produce aflatoxin. Overexpression of veA in the velB mutant only restored conidiation while overexpression of velB in the veA mutant failed to restore either conidiation or aflatoxin production. Yeast two-hybrid assays confirmed that VeA, VelB and LaeA form a complex but suggested that FluG is also likely to be an interacting partner. Concerted interactions of A. flavus VeA and VelB with LaeA are critical for conidiation in the dark and aflatoxin biosynthesis.

310. The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology. S. E. Baker. Environmental Molecular Sciences Laboratory, Pacific Northwest Natl Lab, Richland, WA.

Tools for analysis of classical and reverse genetic mutants play an important role in fungal biology research. The Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory is a US Department of Energy national user facility. EMSL develops and utilizes cutting edge mass spectrometry, NMR, imaging and computational capabilities to accelerate research in a number of areas. We have used EMSL's mass spectrometry capabilities to characterize glycosylation of secreted proteins of *Aspergillus niger*. In addition, we have explored the use of laser ablation and nano-DESI mass spectrometry for spatial localization of molecules associated with *Trichoderma reesei* mycelium. Finally, spores from wildtype and albino strains of *Aspergillus carbonarius* were characterized using helium ion microscopy. As a national user facility, the EMSL is open to the fungal biology community through a competitive, peer-reviewed proposal process.

311. Comparative Genomics and Transcriptomics of Insect Pathogenesis. Kathryn E. Bushley, Joseph W. Spatafora. Dept Botany & Plant Pathology, Oregon State Univ, Corvallis, OR.

We have sequenced the genome of *Tolypocladium inflatum*, the first sequenced representative of one of three major lineages of insect pathogens within the order Hypocreales. Comparisons of the gene space and transcriptome of *T. inflatum* with closely related plant pathogenic and endophytic fungi is providing insights into secondary metabolite arsenals specific to insect pathogens as well as shedding light on shifts in primary metabolism associated with a transition to an insect host. We address the role of secondary metabolites in insect pathogenesis using a combination of comparative genomics to track the evolution of secondary metabolite clusters across the Hypocreales and transcriptomics to characterize patterns of gene expression within metabolite clusters in media supplemented with insect cuticle (simulating insect infection) and hemolymph (simulating insect colonization). We also identify other gene families that are upregulated under these media conditions. GO enrichment analyses of upregulated genes showed that those involved in oxidation-reduction reactions, iron-binding, and transport of iron and inorganic ions are important during both the infection and colonization phases. Genes with serine peptidase and serine hydrolase activity were uniquely upregulated in cuticle media while a large proportion of genes upregulated in hemolymph were involved in transmembrane transport not only of iron, but also of sugars and other carbohydrates. We examine expansions and contractions of some of these gene families (e.g. proteases and P450s) that map to nodes in the phylogeny associated with shifts to insect hosts. We identify patterns that are shared across the three insect pathogenic lineages of Hypocreales versus those which have evolved independently in distinct lineages.

312. Integrated transcriptional profiling and analysis for identification of *Cryptococcus neoformans* genes regulated during human cryptococcal meningitis. Y. Chen[1], J. Tenor[1], D. Toffaletti[1], A. Litvintseva[2], T. Mitchell[1], J. Perfect[1]. 1) Duke University School of Medicine, Durham, NC; 2) Centers for Disease Control and Prevention, Atlanta, GA.

Background: *Cryptococcus neoformans* is an opportunistic fungal pathogen that is the major cause of fungal meningitis in immunocompromised individuals worldwide. Accurate and comprehensive de novo transcriptome profiling of *C. neoformans* in the human host may allow a better understanding of how it survives and produces disease. **Methods and Results:** To identify genes, whose expression is differentially regulated under *in vivo* and *in vitro* conditions, we selected two strains of *C. neoformans var. grubii* (serotype A), which were isolated from the cerebrospinal fluid (CSF) of two AIDS patients from Uganda and the United States. Multilocus sequence typing (MLST) showed that one strain was from VNI clade and one strain from VNII. Next-generation sequencing (RNA-Seq) was used to determine transcriptional profiles of these strains under three conditions: fungal cells were directly taken from CSF of the patients; fungal cells were grown in YPD at 37°C until the stationary phase; fungal cells that reached the stationary phase in YPD were exposed to sterile human CSF for 9 hours. The sequencing results showed that there was no major difference in sequencing quality and contaminations between *in vivo* and *in vitro* samples. Hierarchical clustering analysis revealed that the samples treated with same environment have more similarity in transcriptional profile. Comparative analysis of the expression pattern shows that 144 genes up-regulated in CSF when compared to YPD and 87 genes were up-regulated *in vivo* compared to YPD and 39 genes overlapped between the CSF and *in vivo* condition. Some of the overlapping genes in CSF and *in vivo* have been reported to be related to the virulence composite of *C. neoformans*, such as Rim101 and ENA P-type ATPase 1. Furthermore, we searched for the 100 most divergent expressed genes between the two strains. Gene Ontology (GO) term enrichment analysis showed an enrichment of GO terms in transporter activity between the strains. **Conclusion:** We provide the first transcriptome profiling of *C. neoformans* taken directly from the CSF of two human patients. The comparisons between in vivo and in vitro samples helped us to identify a group of genes that may be important for surviving, adapting and proliferating of *C. neoformans* in the CSF of the human host.

313. Structural and functional characterization of microRNA-like RNAs in the penicillin producing fungus *Penicillium chrysogenum*. Tim Dahlmann, Minou Nowrousian, Ulrich Kück. Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Deutschland, tim.dahlmann@rub.de.

MicroRNAs are endogenous RNAs with a size of about 22 nt and post-transcriptionally regulate gene expression in metazoan and plants. MicroRNAs are derived from RNA hairpin precursors, which are usually transcribed by RNA polymerase II. Recent studies on small RNA binding components of the RNA-induced silencing complex (RISC) show the existence of microRNA-like RNAs (milRNAs) in *Neurospora crassa*, which give a first hint of a post-transcriptional regulatory mechanism based on microRNAs in fungi [1]. So far only little is known about microRNA-like molecules in other fungi, especially about their role in fungal development and gene regulation.

To investigate the occurrence of milRNAs and their involvement in gene regulation in the penicillin producing fungus *Penicillium chrysogenum*, we performed predictions of putative microRNAs. Therefore small RNAs (19 - 50 nt) representing different growing conditions and developmental stages, were used for RNA next generation sequencing. The calculation of putative microRNA precursors was performed with the program miRDeep [2], and is based on the distribution of RNA sequence reads in afore predicted RNA hairpin molecules. By this approach, we were able to identify structures, which show the typical characteristics of microRNA precursors. To confirm the *in silico* predictions, transcript analyses were performed. These analyses support the existence of small RNAs and their precursors and show various expression pattern of the putative milRNAs under different growing conditions. To investigate the regulatory role of the identified milRNAs, strains lacking or overexpressing milRNAs were generated. In addition, we have constructed artificial microRNAs to investigate their use as molecular genetic tools to mediate gene specific RNA interference (RNAi). The results of this study provide evidence for milRNAs in *P. chrysogenum* and indicate a milRNA based silencing mechanism in this fungus.

[1] Lee HC et al. (2010) Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. Molecular Cell 38:803-814
[2] Friedländer MR et al. (2008) Discovering microRNAs from deep sequencing data using miRDeep. Nat Biotechnol 26:407-415.

314. Metatranscriptomic analysis of ectomycorrhizal root clusters in *Pinus taeda*: new methodologies for assessing functional gene expression *in situ*. H.-L. Liao[1], Y. Chen[2], T. D. Bruns[3], K. G. Peay[4], J. W. Taylor[3], S. Branco[3], J. M. Talbot[4], R. Vilgalys[1]. 1) Department of Biology Duke University, Durham, NC; 2) School of Medicine, Duke University, Durham, NC; 3) Department of Plant and Microbial Biology, UC-Berkeley, Berkeley, CA; 4) Department of Biology, Stanford University, Stanford, CA.

A highly diverse community of ectomycorrhizal (ECM) fungi are known to associate with members of the genus *Pinus*. Less is known about how diverse fungal communities affect functional diversity within ECM roots. Here we present an optimized method for metatranscriptomic analysis of the ECM-pine root interaction in a natural system. RNA was purified using a CTAB method from individual ECM root clusters collected at varying spatial scales across the distribution range of *P. taeda*, and sequenced using Illumina HiSeq technology. About 35 million qualified reads were obtained. Sequences were initially assembled using reference based mapping (Bowtie) to sort the reads that represent rRNA from fungal and bacterial species. Reads from divergent regions (D1-D2) of fungal LSU rRNA were used to identify dominant ECM and other fungal community members. Subsequently, *P. taeda* genes and functional genes of dominant fungal species were sorted using public cDNA databases. The Trinity package was used for *de novo* assembly of un-mapped reads (mostly fungal genes). Blastx and Go packages were used for gene annotation. A typical ECM root cluster was found 45% *P. taeda* genes, 3% fungal rRNA, 0.05% bacterial 16S rRNA, 30% fungal functional genes, 10% unknown sequences, and 12% unassembled reads. Analysis of D1-D2 LSU sequences confirmed that a single ECM fungal species usually dominates individual root clusters. *De novo* assemblies of fungal genes yielded 120 thousand contigs from 10 million reads representing 90 thousand unique genes with highly similarity to known ECM fungi. Functional analysis revealed that most of the transcripts recovered were involved with translation, protein degradation, heat shock, superoxide metabolism, electron transfer, signaling, and C/N metabolism. Highly expressed transcripts recovered from *Piloderma*, which was abundant in our samples, included genes encoding a wide array of metabolic enzymes: chitosanase, phosphatase, glutamine synthetase, terpene synthases, b-glucanase; transporters for P+ and oligopeptides; cell signaling: calmodulin, cAMP-regulated phosphoprotein (Igo1); C/N related genes: lectin, cross-pathway control (cpc1); as well as several genes with unknown function. Future studies will seek to address how ECM metatranscriptomes change in response to different *Pinus* hosts and across different spatial scales.

315. Transcriptomic response of *Neurospora crassa* germinating conidia to chitosan in sub-lethal dose. Federico Lopez-Moya[1], David Kowbel[2], N. Louise Glass[2], Luis Vicente Lopez-Llorca[1]. 1) Laboratory of Plant Pathology, Multidisciplinary Institute for Environment Studies (MIES) Ramón Margalef. University of Alicante, Alicante, SPAIN; 2) Department of Plant and Microbial Biology, University of California, Berkeley CA, 94720-3120 USA.

Chitosan is a natural polymer able to permeabilize *Neurospora crassa* membranes, in an energy dependent manner. Plasma membrane permeabilisation by chitosan depends on membrane fluidity, with FFA unsaturated membrane fungi (*N. crassa*) being chitosan sensitive, the plasma membrane fluidity is an

important factor in chitosan effect. Plasma membrane permeabilisation is also enhanced under starvation conditions, also causes intracellular ROS increases and is involved in cell division. Conidial germination is the most sensitive step to chitosan in filamentous fungi. We have used *Neurospora crassa* conidia germinating in Vogels medium with chitosan at a sub-lethal concentration for evaluating the transcriptomic response of the fungus. We determined chitosan IC_{50} for *N.crassa* (4, 8 and 16 hours after inoculation, hai) and analyzed the effect on gene expression over time. Using RNA-seq we have detected the genes involved in *N. crassa* response to chitosan, we analyzed with bioinformatic resources the expression involved on chitosan response in *N. crassa* conidia . We have also re-annoted the complete *Neurospora crassa* OR74A genome (Broad Institute) for allowing GO analyses (biological process, cell component and molecular function) of our RNA-seq data. Chitosan early induced (4-8 hai) some genes, involved in ROS protection (e.g. catalases, monooxigenase and SOD) and also in nitrogen compounds transporter and degradation. We also detected late expression of genes involved in biosynthetic processes, nitrogen compounds (including proteins) metabolism and transport. Our strategy has allowed us to gain an important insight on chitosan mode of action. This will open new possibilities for application this versatile natural compound.

316. Transcriptomic analysis of the interaction between Trichoderma harzianum and the phytopathogen Sclerotinia sclerotiorum using the RNA-seq approach. Andrei S. Steindorff[1], Marcelo H.S. Ramada[1], Robert Miller[1], Georgios J. Pappas[1], Cirano J. Ulhoa[2], Eliane F. Noronha[1]. 1) Cell Biology Dept, Brasilia University, Brasilia, DF, Brazil; 2) Biochemistry Dept, Federal University of Goias, Goiania, Brazil.

The plant pathogen Sclerotinia sclerotiorum is the causal agent of the common bean's (Phaseolus vulgaris) root rot disease, white mold, and its occurrence is responsible for the great yield losses in irrigated areas of the Southeast and Midwest regions of Brazil. Species of the fungi genus Trichoderma have been used in the biological control of this pathogen as an alternative to chemical control. To gain new insights into the biocontrol mechanism used by Trichoderma harzianum against the phytopathogenic fungus, Sclerotinia sclerotiorum, our research group performed a transcriptomic analysis of this interaction using RNA-seq and quantitative real-time PCR (RT-qPCR) approaches. Six RNA-seq libraries from T. harzianum mycelium (isolate 303/02) grown on cell walls of S. sclerotiorum (CWSS) and glucose during 12, 24 and 36 h were constructed, sequenced by Illumina HiSeq 2000 2x100pb and analyzed by TopHat/Cufflinks pipeline. The T. harzianum CBS 226.95 v1.0 genome (http://genome.jgi.doe.gov/Triha1/Triha1.home.html) was used as a reference for the bioinformatics analysis. Among the 13616 genes mapped, 1581 genes were found differentially expressed among the growth in the presence of glucose or plant pathogen cell walls. Moreover, the expression pattern was also time course dependent. Transporters, fungal cell wall hydrolases, peptidases, transcriptional factors and proteins presenting role in the environmental interaction were found high expressed by the Trichoderma isolate in the three different times of growth and in the presence of the pathogen. Some genes with no known functions were also found. The role of cell wall hydrolases, peptidases and other hydrolytic enzymes in the mycoparasitism by Trichoderma species was strongly recorded, therefore the description of the functions of those unknown functions genes in biocontrol will orientate future works. Indeed, the present work will contribute to an initial mapping of the transcripts quite related to the interaction among these two fungi and for its further analysis under in vivo interaction.

317. Genome evolution of the original Saccharomyces carlsbergensis lager yeast strain, Unterhefe No1, as revealed by whole genome sequencing. Andrea Walther, Ana Hesselbart, Jürgen Wendland. Carlsberg Laboratory, Copenhagen V, Denmark.

The first lager beer yeast strain was purified by Hansen in 1883 who termed this original strain Unterhefe No1, also known as Saccharomyces carlsbergensis. It became evident that Unterhefe No1 is a hybrid between two closely related Saccharomyces species, particularly S. cerevisiae and a S. bayanus-like strain. We have compared key fermentation parameters including sugar utilization, ethanol and flavor production of Unterhefe No1 with currently used industrial lager yeast strains. We then sequenced the S. carlsbergensis genome using 454 next generation sequencing methods and determined its hybrid genome content. We found that the genome has evolved from a presumed ancestral tetraploid cell as a result of adaptation to brewing conditions resulting in its current allotetraploid state. In contrast to a hypothetical tetraploid genome with 24Mb unique DNA contributed by its parents and distributed on 32 chromosomes, the Unterhefe No1 genome consists of only 20.7Mb. It comprises chromosomes derived from S. cerevisiae and a non-cerevisiae parental strain as well as a S. bayanus like mitochondrial genome. In total, we found 29 different chromosomes including evolved chromosomes displaying several events of loss of heterozygosity and massive chromosomal rearrangements. Comparison of the genome of S. carlsbergensis with other yeast genomes provides insight into the evolution of this brewing strain as a consequence of adaptation to lager beer fermentation conditions.

318. Retention of genes in a secondary metabolite gene cluster that has degenerated in multiple lineages of the Ascomycota. Daren W. Brown[1], Hege H. Divon[2], Erik Lysøe[3], Robert H. Proctor[1]. 1) Bacterial Foodborne Pathogens and Mycology Research, USDA/ARS, Peoria, IL; 2) Section of Mycology, Norwegian Veterinary Institute, PO Box 750, Sentrum, 0106 Oslo, Norway; 3) Department of Plant Health and Plant Protection, Bioforsk - Norwegian Institute of Agricultural and Environmental Research, 1432 Ås, Norway.

Fungal secondary metabolite (SM) gene clusters encode proteins involved in SM biosynthesis, protection against SMs, and regulation of cluster gene transcription. RNA-Seq analysis of *Fusarium langsethiae* (class Sordariomycetes) revealed a cluster of six genes that were highly expressed during growth in oat-grain medium, but not in complete medium. All six genes share significant homology and synteny with genes in the *Alternaria brassicicola* (class Dothideomycete) cluster responsible for production of the SM depudecin. HPLC analysis confirmed the presence of depudecin in oat-grain medium and absence from complete medium cultures. A survey of publically available genome sequences identified eight complete and 14 partial depudecin biosynthetic gene (*DEP*) cluster homologs in fungi across distantly related classes of Ascomycota. Most of the partial clusters included pseudogenes due to single nucleotide changes and/or multiple nucleotide deletions, indicating that the partial clusters are derived by degeneration of complete clusters. Most of the partial clusters also included apparently functional homologs of the major facilitator superfamily (MFS) transporter (*DEP3*) and transcription factor (*DEP6*) genes. Retention of these two genes may provide a defense mechanism against depudecin produced by other fungi. Alternatively, *DEP3* and *DEP6* in the partial clusters may have been repurposed to provide a selective advantage different from the advantage conferred by depudecin. The shared synteny of putative functional *DEP3* and *DEP6*, as well as phylogenetic analysis of these genes, suggest that the *DEP* cluster has been transferred horizontally between fungi multiple times.

319. Functional characterization of unique non-ribosomal peptide synthetase genes in the cereal fungal pathogen *Cochliobolus sativus*. Yueqiang Leng, Shaobin Zhong. Department of Plant Pathology, North Dakota State University, Fargo, ND 58108.

In filamentous fungi, nonribosomal peptide synthetases (NRPSs) are the major enzymes involved in biosynthesis of nonribosomal peptides (NRPs), some of which have been demonstrated to be involved in pathogenicity or virulence of fungal plant pathogens. However, the functions of many genes (*NPS*) encoding NRPSs are still not well understood. We identified 25 *NPS* genes from the genome sequence of the cereal fungal pathogen *Cochliobolus sativus*. Genome comparison among species in the genus of *Cochliobolus* identified 14 unique *NPS* genes in *C. sativus* with five (encoding protein ID# 130053, 140513, 104448, 115356 and 350779, respectively) being unique to the pathotype 2 isolate ND90Pr of the fungus. Quantitative real time PCR revealed that

all these unique *NPS* genes of ND90Pr except the one for ID# 350779 were highly up-regulated in planta 12 hours post inoculation on barley cv. Bowman. Knockout mutants of the *NPS* gene for ID# 115356 and RNAi mutants of the *NPS* gene for ID# 140513 were significantly reduced in virulence on Bowman, but they had the same morphology and growth rate under the conditions of normal growth and oxidative/hyperosmotic stresses compared to the wild type. These results indicate that these *NPS* genes are required for the high virulence of the pathotype 2 isolate on barley cv. Bowman. Functional characterization of other unique *NPS* genes of ND90Pr will also be presented.

320. Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales. C. Alisha Owensby, Kathryn E. Bushley, Joseph W. Spatafora. Botany & Plant Pathology, Oregon State University, Corvallis, OR.

Hypocreales is an order characterized by a dynamic evolutionary history of interkingdom host jumping, with members that parasitize animals, plants, and other fungi. The monophyly of taxa attacking members of the same kingdom is not supported by molecular phylogenetics, however. For example, *Trichoderma spp.* and *Elaphocordyceps spp.* are both mycoparasitic, but are members of different families within Hypocreales, Hypocreaceae and Ophiocordycipitaceae, respectively. In fact, both genera are more closely related to insect pathogens, than they are to each other. Multiple species of *Trichoderma* have sequenced genomes, and recently genomes of several insect pathogens in Hypocreales have been completed (e.g. *Metarhizium spp.* and *Tolypocladium inflatum*). The genus *Elaphocordyceps* represents a unique clade within Hypocreales, because whereas most species in the family Ophiocordycipitaceae are insect pathogens, most *Elaphocordyceps* parasitize truffles of the ectomycorrhizal genus *Elaphomyces* [Eurotiales, Ascomycota]. To compare genes of a truffle pathogen with hypocrealean insect pathogens and mycoparasites, we sequenced the genome of *Elaphocordyceps ophioglossoides*. Our draft assembly of the *E. ophioglossoides* genome is ~32 MB and has 10,779 gene models, 36 of which are predicted to produce secondary metabolites. We have identified three very large genes in *E. ophioglossoides* related to peptaibol producing nonribosomal peptide synthetase (NRPS) genes. Peptaibols, which disrupt osmoregulation by forming ion channels through lipid bilayers, have antibiotic and antifungal activity and are best described in *Trichoderma spp*. *E. ophioglossoides* and its beetle-pathogenic congener, *T. inflatum*, both possess three putative peptaibol synthetases which we identified through analysis of NRPS adenylation domains. Of the three peptaibol-specific domain clades, one is predicted to encode for the nonproteinogenic a-aminoisobutryic acid residues. We also show that, despite being very closely related, *E. ophioglossoides* and *T. inflatum* each possess three different peptaibol-like genes, only two of which appear to be located in syntenic regions. The current distribution of fungi possessing peptaibol genes is restricted to mycoparasitic lineages of Hypocreales and is generating hypotheses about the role of secondary metabolites in mycoparasitism.

321. Genome and transcriptome sequence of the apomictic fungus *Arnium arizonense* (*Podospora arizonensis*). E. Coppin[1,2], C. Drevet[3], L. Peraza-Reyes[1,2], D. Zickler[1,2], E. Espagne[1,2], J. Aït-Benkhali[1,2], P. Silar[1,2,4], A. E. Bell[5], D. P. Mahoney[5], R. Debuchy[1,2]. 1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) Univ Paris-Sud, eBio bioinformatics plateform, Orsay France; 4) UFR des Sciences du Vivant, Université Paris-7 Diderot, Paris, France; 5) Private Mycological Research, 45 Gurney Road, Lower Hutt, New Zealand.

The homothallic fungus *Arnium arizonense* is closely related to the heterothallic *Podospora anserina* but displays several unique features. It is apomictic, *i.e*. dikaryotic croziers are formed inside the perithecia but neither karyogamy nor meiosis take place in the asci, although morphological changes in both chromosomes and spindle pole bodies are reminiscent of those associated with meiosis in heteromictic Pezizomycotina. Instead of meiosis, the two nuclei undergo two mitoses and the resulting eight nuclei are enclosed in uninucleate ascospores, among which four mature normally, and four abort. Arrangement of the two ascospore types in individual asci is random (Mainwaring and Wilson, 1968, Trans Br mycol Soc, 51, 663). *A. arizonense* has two chromosomes, while most fungi in this group have seven chromosomes. Analysis of the genome sequence revealed that *A. arizonense* contained linked counterparts of the *P. anserina* mating-type genes, a structure that is typical of homothallic life style. Deletion of the mating-type locus resulted in the loss of perithecium formation, thus confirming the role of the mating-type genes in the fruit-body development. Genome annotation identified 11,165 genes, of which 476 undergo alternative splicing. Comparison of *A. arizonense* proteins with their orthologs in *P. anserina* revealed that *A. arizonense* genome contains numerous pseudogenes. Direction for future work is to determine how apomixis takes place, as this process of asexual clonal reproduction through seeds has potential revolutionary applications in agriculture by allowing perpetuation of any important selected heterozygous genotype (reviewed by Ozias-Akins and van Dijk, 2007, Ann Rev Genet, 41, 509-537).

322. Role of MAP kinase pathways in the pathogenicity of the wheat pathogen *Mycosphaerella graminicola* . Elisabetta Marchegiani[1], Julie Vallet[1], Siân Deller[2], Marc-Henri Lebrun[1]. 1) Bioger, INRA, Thiverval-Grignon, France; 2) Syngenta Limited, European Regional Centre, Priestley Road, Surrey Research Park, Guildford, Surrey, GU2 7YH, United Kingdom.

Mitogen-activated protein kinases (MAPKs) are essential components of fungal signaling pathways involved in different developmental processes and are required for host plant infection. *Mycosphaerella graminicola*, the causal agent of *Septoria tritici* leaf blotch (STB) of wheat, has three MAPK pathways that are all required for infection (MgFUS3 , MgHOG1, MgSLT2; Cousin et al., 2006; Mehrabi et al., 2006a, Mehrabi et al., 2006b). We showed that *Mgfus3* null mutants are non-pathogenic on intact wheat leaves (paint brush inoculation), but highly-reduced in pathogenicity when infiltrated into leaf tissues by syringe injection (reduced necrosis, low number of pycnidia). This suggests that *MgFUS3* is involved in fungal penetration, host colonization and pycnidia formation. *Mghog1* null mutants have pathogenicity defects similar to *Mgfus3* null mutants. This result highlights that the role of HOG1 in pathogenicity on plants differs among fungi (Segmüller et al., 2007). *Mgslt2* null mutants are fully non-pathogenic on inoculated wheat leaves either by paint brush inoculation or injection. This phenotype is unusual among slt2 null mutants from other fungi. Therefore, *Mycosphaerella graminicola* MAPK pathways may have evolved to control regulatory networks differing from other fungal plant pathogens. To identify which genes are under the control of the *MgSLT2* signaling pathway, we are developing different transcriptomics analyses. Expression profiling relies on the comparison of transcriptomes of *Mgslt2* null mutants and wild type strains grown under conditions corresponding to either an active or an inactive SLT2 pathway. Additional transcriptomics analyses will be performed using an allele encoding a conditionally active MAPKK expressed under the control of an inducible/repressible promoter. Genes whose expression requires an active SLT2 MAPK will be further studied for their role in development and infection using reverse genetics. Cousin et al. (2006), Molecular Plant Pathology 7(4): 269-278. Mehrabi et al; (2006a), Molecular Plant-Microbe Interactions 19(4): 389-398; Mehrabi et al. (2006b), Molecular Plant-Microbe Interactions 19(11): 1262-1269; Segmüller et al. (2007), Eucaryotic Cell 6(2) 211-221.

323. WITHDRAWN

324. Ancient and abundant MITEs in epichloae genomes. Damien Fleetwood[1], Chris Schardl[2], Carolyn Young[3]. 1) Forage Improvement Section, AgResearch, Auckland, New Zealand; 2) Dept of Plant Pathology, University of Kentucky, Lexington, KY; 3) Forage Improvement Division, Samuel Roberts Noble Foundation, Ardmore, OK.

The *Epichloë festucae* genome contains thirteen known degenerate miniature inverted repeat transposable element (MITE) families that make up almost 1% of the genome. Recent sequencing of a range of epichloae and related Clavicipitaceae family genomes revealed that every MITE family was active early in the evolution of the epichloid lineage although none are found in other closely related genera. Analysis of MITE integration sites showed that these elements have a target integration site preference for 5' genic regions of the *E. festucae* genome and are particularly enriched within alkaloid gene clusters and within 10-kb of other NRPS and PKS genes. Very few individual insertion sites are apparently shared among different species although one ancestral insertion - three adjacent EFT-3m/Toru elements in the ergot alkaloid synthesis cluster - has mediated recombination events that in one strain may have abolished synthesis of this bioprotective alkaloid. Overall these results suggest a potential role for MITEs in the evolution of the epichloae and their symbiotic associations with plants.

325. Exploring the biomass modifying enzymes of new filamentous fungal isolates from Vietnam, using secretome and transcriptome analyses. George E Anasontzis[1,3], Thanh Dang Tat[2], Thuy Nguyen Thanh[2], Hang Dinh Thi My[2], Thanh Vu Nguyen[2], Lisbeth Olsson[1,3]. 1) Industrial Biotechnology, Chalmers University of Technology, Gothenburg, Västra Götaland, Sweden; 2) Department of Microbiology, FIRI - Food Industries Research Institute, Hanoi, Vietnam; 3) Wallenberg Wood Science Center, Chalmers, Gothenburg, Sweden.

In the bio-based economy concept, the current hydrocarbon fuels and non-biodegradable plastics will be replaced by new products which will derive from natural and renewable resources. The synthesis of such biofuels and biochemicals is still challenged by the difficulties to cost efficiently degrade lignocellulosic materials to fermentable sugars or to isolate the intact polymers. Biomass degrading and modifying enzymes play an integral role both in the separation of the polymers from the wood network, as well as in subsequent modifications, prior to further product development. The type of application usually defines the conditions where the reactions should take place. Thus, novel enzymes with variable combined properties, such as different thermotolerance, pH range of activity, substrate specificity and solvent tolerance, still need to be discovered and developed to achieve the highest possible efficiency in each occasion. We took advantage of the rapidly evolving and high biodiversity of the tropics and have been screening various isolates for their cellulases and hemicellulases activities. Promising strains were then cultivated in bioreactors with different carbon sources, such as wheat bran, spruce and avicel and their biomass degrading capacity was analysed through cross species protein identification of their secretome with iTRAQ. Information on the genes involved in the different stages of the fermentation and the carbon source are being acquired with next generation sequencing of the total transcriptome. Interesting transcripts will then be used to heterologously clone and express the respective genes and identify their role in the degradation process.

326. *Fusarium* Comparative Transcriptomics and Transcriptional Regulatory Network Reconstruction. L. Guo[1], G. Zhao[2], X. Zhao[3], W. Jonkers[4], L. Gao[2], J. Xu[3], C.H. Kistler[4], L. Ma[1]. 1) Comparative Fungal Genomics Laboratory, University of Massachusetts Amherst, Amherst, MA; 2) Department of Electrical & Computer Engineering, University of Massachusetts Amherst, Amherst, MA; 3) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN; 4) USDA-ARS, Cereal Disease Laboratory, St Paul, MN.

Genus *Fusarium* contains pathogens that infect hundreds of crop plants as well as humans and thus threatens global food safety and human health. As in other cellular organisms, diseases caused by this group of organisms are dynamically controlled through their transcriptional regulatory networks (TRNs). Reconstructing their TRNs will not only help us to comprehend the complexity of their cellular functions, but will also have broad implications for disease management and prevention. A robust searching algorithm using Bayesian networks model was developed based on nearly 200 gene expression datasets of *F. graminearum*. The algorithm infers the relationship between candidate regulators (transcription factors and signaling proteins) and the target genes regulated by them. Preliminary validation of the inferred network using prior biological knowledge proofs the effectiveness of the program. Using comparative functional genomics approach, we have analyzed the microarray-based transcriptome data of *F. graminearum* (PH1), *F. verticillioides* (7600) and *F. oxysporum* f.sp. *lycopersici* (4287) in response to carbon (C) and nitrogen (N) starvation. In agreement with previous studies, under C and N starvation, fungal cells adjust to extreme environments via modulating expression of core orthologous genes to enhance cellular transport of lipid, peptide and carbohydrates but shut down unnecessary energy consumption such as protein synthesis. This analysis helps us to reach the understanding of functional conservation of the orthologs, judging by their expression under the same biological condition in different species. Even though there is not equal amount of expression data for other *Fusarium* spp., the conservation of the regulatory modules will enable us to transfer the network knowledge from one system to improve the prediction of the other. The comparative functional analysis will also highlight critical pathways that constitute to species-specific phenotypes, such as pathogenicity in each species.

327. The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq. A. Kohler[1], E. Tisserant[1], E. Morin[1], C. Veneault-Fourrey[1], S. Abba[2], F. Buscot[3], J. Doré[4], G. Gay[4], M. Girlanda[2], S. Herrmann[3], T. Johansson[5], U. Lahrmann[6], E. Martino[2], S. Perotto[2], M. Tarrka[3], A. Tunlid[5], A. Zuccaro[6], I. Grigoriev[7], F. Martin[1]. 1) Lab of Excellence ARBRE, Tree-Microbes Department, INRA-Nancy, Champenoux, France; 2) Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino,Torino, Italy; 3) Department Soil Ecology, UFZ Centre for Environmental Research Leipzig-Halle Ltd., Halle, Germany; 4) Ecologie Microbienne UMR CNRS 5557, USC INRA 1193, Universite Claude-Bernard LYON 1, Villeurbanne, France; 5) Microbial Ecology, Lunds University, Lund, Sweden; 6) Max-Planck Insitute for Terrestrial Microbiology, Marburg, Germany; 7) DOE Joint Genome Institute, Walnut Creek, California, USA.

Genome and transcriptome analyses of *Laccaria bicolo*r and *Tuber melanosporum* (Martin *et al.*, 2008, 2010) revealed that the ectomycorrhizal symbiosis probably developed several times during evolution by generating different 'symbiosis molecular toolkits'. In *L. bicolor* a large set of small-secreted proteins acts as putative effectors but not in T. *melanosporum*, while the up-regulation of transporter-coding genes seems to be a common feature of both interactions. To better understand the evolutionary origin of mycorrhizal symbiosis and to elucidate the molecular mechanisms involved, a large sequencing project of species from different taxa, phylogenetic clades and symbiotic lifestyles (ectomycorrhizae, ericoid and orchid mycorrhizae) was started in 2011 by the Joint Genome Institute and the mycorrhizal genome initiative. To identify and to compare symbiosis-regulated genes large scale Illumina transcriptome sequencing of mycelium and mycorrhizal roots from *Paxillus involutus, Piloderma croceum, Hebeloma cylindrosporum, Sebacina vermifera, Tulasnella calospora* and *Oidiodendron maius* was performed. Small-secreted proteins, transporters, CAZymes but also many lineage specific proteins were among the highly up-regulated transcripts.

Martin, F., Aerts, A., Ahrén, D., Brun, A., Duchaussoy, F., Kohler, A., et al. 2008. The genome sequence of the basidiomycete fungus Laccaria bicolor provides insights inot the mycorrhizal symbiosis. Nature 452 :88-92

Martin, F., Kohler, A., Murat, C., Balestrini, R., Coutinho, P.M., Jaillon, O., Montanini, B., et al. 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464 :1033-1038.

328. Transcriptome, secreted enzymes and systematics of the white rot basidiomycete *Phlebia radiata*. Jaana Kuuskeri[1], Miia Mäkelä[1], Kristiina Hildén[1],

Pia Laine[2], Lars Paulin[2], Taina Lundell[1]. 1) Department of Food and Environmental Sciences, Division of Microbiology, Fungal Biotechnology Laboratory; 2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, Viikki Campus, University of Helsinki, FINLAND.

The efficient wood-degrading white-rot basidiomycete *Phlebia radiata* Fr. is able to degrade all the main components of lignocellulose and secretes a repertoire of lignin-converting peroxidases and oxidases as well as carbohydrate-acting enzymes. *P. radiata* is the second species of the genus *Phlebia* to be genome sequenced, thus giving more insight in to the phlebioid clade of the order Polyporales, class Agaricomycetes, and comparative data for functional analyses on white-rot fungal mechanisms of wood decay and decomposition of lignin. We carried out transcriptome sequencing and secretome analyses on the Finnish isolate 79. Molecular systematics of the genus *Phlebia* was inferred by perfoming a four-gene study on North-European *Phlebia* spp. isolates including 10 species. Ribosomal RNA-encoding (SSU 18S rDNA; ITS1-5.8S-ITS2; ITS2-28S) regions and two protein-coding genes (*gapdh, rpb2*) were partially PCR-amplified with fungal or basidiomycete-specific primer pairs. Phylogenetic sequence analyses resulted with no single taxonomic cluster of *Phlebia*. The genus is obviously polyphyletic, and the various species were scattered together with other genera of Polyporales, like *Phanerochaete* and *Rhizochaete*, in the families Corticiaceae and Meruliaceae. *P. radiata* was the most related with *Phlebia acerina* and *P. rufa* along with *P. tremellosa* and *P. brevispora*, whereas *P. subserialis* and *Phlebiopsis gigantea* are more distant. *P. radiata* transcriptome analysis resulted with 6 590 unique gene transcripts, and similarity searches with blastx (E-value cut-off 1e-6) matched 77% of the unique transcripts to known or predicted protein-coding gene sequences. Functional annotation assigned Gene Ontology term for 54% of the gene transcripts. Most of the genes were annotated with term nucleotide binding whereas 7% were oxidoreductases. Among these were several lignin-modifying enzymes (class II heme-including peroxidases and laccases). From the dataset, 16% of the gene transcripts were unknown thus representing proteins potentially unique to *P. radiata*. Also, the mitochondrial genome of over 150 kb in size shows unique features and high degree of genetic flexibility. These results provide an insight into gene content of *P. radiata* and genome-level transcriptional information on fungal genetic machinery for growth on complex liquid media.

329. Characterization of molecular mechanisms underlying the multi-drug-resistant phenotypes of *Mycosphaerella graminicola* field isolates. Selim Omrane[1], Anne-Sophie Walker[1], Hind Sghyer[1], Catherine Lanen[1], Lamia Aouini[2], Gert Keema[2], Sabine Fillinger[1]. 1) BIOGER, INRA, Thiverval-Grignon, France; 2) Plant Research International, Wageningen University, Wageningen, The Netherlands.

Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs used against them. The basic MDR mechanism is relying on an overexpressed efflux transport system that actively expulses the toxic agent outside the cell. In fungi, MDR (or PDR) has been extensively studied in *Saccharomyces cerevisiae* and *Candida albicans*, but also plant pathogenic fungi, e.g., *Botrytis cinerea, Oculimacula yallundae* and *Mycosphaerella graminicola* are concerned by this phenomenon. In agriculture, it is currently under investigation if MDR strains may threaten the efficacy of current fungicide treatments. MDR strains were detected in septoria leaf blotch (*M. graminicola*) field populations since 2008. These strains are slightly more resistant to DMI (inhibitor of the sterol 14 a-demethylase) fungicides than comparable *cyp51* genotypes and cross-resistant to fungicides with different modes of action. The identification of the molecular mechanism explaining the MDR phenotype in two isolated strains (MDR6 and MDR7) was the main goal of this study. By the use of C14-prochloraz, a DMI, we demonstrated increased fungicide efflux in both MDR strains in comparison to sensitive strains. RNA-sequencing led to the identification of several overexpressed transporter genes, out of which one MFS (major facilitator family) transporter had particularly abundant mRNA in both MDR strains. Crosses between both MDR strains showed that *mdr6* and *mdr7* loci are closely linked. We applied bulk-progeny sequencing to progeny of the crosses MDR6 x sensitive and MDR7 x sensitive in order to map the genomic regions co-segregating with the MDR phenotypes. SNP frequency analysis in sensitive and resistant bulks showed a clear co-segregation between phenotypes and the left arm of chromosome 7. This region harbors a gene cluster including the MFS transporter gene mentioned above. After sequencing the *mfs* promoter, we identified a 514 bp insertion in both MDR strains. Further studies are needed to validate the role of this insertion leading putatively to mfs overexpression and to clarify its relation to the MDR phenotype in the two studied strains. Financial support: Arvalis Institut du Vegetal, BASF Agro SAS, Bayer SAS, DuPont de Nemours SAS, Syngenta Crop Protection AG.

330. Candidate pathogenesis gene identification via *Ustilago maydis* `first gen` genomic analyses. M.E. Donaldson[1], S. Meng[2], B.J. Saville[1,3]. 1) Environmental & Life Sciences, Trent University, Peterborough, Ontario, Canada; 2) Lineberge Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, N.C., USA; 3) Forensic Science Program, Trent University, Peterborough, ON, Canada.

Three different approaches were used to identify candidate pathogenesis genes for the model plant pathogen *Ustilago maydis* (DC) Corda: 1) suppressive subtractive hybridization (SSH) cDNA library analysis, 2) a bioinformatics approach, selecting genes unique among pathogenic basidiomycete fungi, and 3) microarray hybridization analyses. The SSH cDNA library was constructed to capture *U. maydis* genes expressed *in planta*, as well as *Zea mays* genes up-regulated during the infection process. The resulting ESTs represented 23 *U. maydis*, and 159 *Zea mays* transcripts, respectively. Analysis of the *U. maydis* transcripts revealed 14 genes which have been previously characterized, 8 genes of unknown function, and 1 putative non-coding RNA. The fact that not all of the transcripts code secreted proteins is consistent with the observation by others that not all *U. maydis* effectors have recognizable secretion signals. RT-PCR results supported *in planta* transcript levels for a subset these genes. To evaluate the three strategies, one gene from each series of experiments was chosen for selective gene deletion experiments. Deletion strains were created for: 1) a hypothetical gene (*um03046*) that had high representation in the SSH cDNA library, 2) a conserved hypothetical gene (*um01632*) unique among basidiomycetes, and 3) the calcineurin B regulatory subunit (*cnb*, *um10226*), identified through microarray hybridization as two-fold more highly expressed in the dikaryotic filamentous growth form compared to the diploid filamentous form. All *U. maydis* deletion strains were capable of mating on DCM medium containing charcoal. Mutant *U. maydis* clones disrupted for *um03046* and *um01632* did not show aberrant growth phenotypes; and the morphology of these cells was indistinguishable from wild-type strains. In contrast, the *cnb* mutants did not appear to separate after budding. In pathogenesis assays, the *um03046* and *um01632* mutants were slightly more, or less pathogenic than wild-type infections, respectively. Results for the *cnb* mutants were more striking, with a marked 77% decrease in the disease index, compared to wild-type infections. Together, these results support SSH cDNA library and microarray hybridization analyses as useful tools in identifying genes expressed during specific stages of *in planta* development and those genes involved in pathogenesis.

331. A biocontrol agent among pathogens : How *Pseudozyma flocculosa* genome relates to singular lifestyle. F. Lefebvre[1], D.L. Joly[2], G. Bakkeren[2], F. Belzile[3], R.R. Bélanger[1]. 1) Centre de recherche en horticulture, Département de phytologie, Université Laval, Quebec, QC, Canada; 2) Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada; 3) Institut de biologie intégrative et des systèmes, Département de phytologie, Université Laval, Québec, QC, Canada.

Most fungal species belonging to the Ustilaginales are well known for their pathogenic activity towards a variety of plant species. One interesting exception is *Pseudozyma flocculosa* that rather acts as a biocontrol agent against powdery mildews. In order to better understand the factors underlying these opposed lifestyles among closely related organisms, the genome of *P. flocculosa* was first sequenced and annotated on the basis of homology to

known protein sequences, RNA-Seq data and *ab initio* predictors. Then, based on comparative genomics with the pathogenic species *Ustilago maydis*, *Ustilago hordei* and *Sporisorium reilianum*, we identified key features that could explain both the avirulent nature of *P. flocculosa* toward plants and the virulent features of the pathogenic Ustilaginales.

First, the genome structural annotation showed similarities in total gene number, gene density and average gene length. But these similarities hides major differences with regards to average intron per gene and GC content. In fact, *P. flocculosa* has about 4 times more introns than *U. maydis* and has a very high GC content of 65.1%. Moreover, *P. flocculosa* genome shows a lower level of synteny than pathogenic species compared together.

Second, comparison of gene content revealed unexpected results. On one hand, the genome of *P. flocculosa* harbor many traits usually associated with pathogenic species like plant cell-wall degrading enzymes and genes involved in the synthesis of secondary metabolites that are highly conserved compared to pathogenic species. On the other hand, genes coding for candidate secreted effector proteins (CSEPs) show a much lower level of conservation, that seem to explain, in part, the differences in lifestyle.

Finally, this work led to the identification of certain of the most interesting genetic features in the study of pathogens and biocontrol agents.

332. A tale of two poplar pathogens - Moving from sequence to function. B. Dhillon[1], N. Feau[1], P. Tanguay[2], M. Sakalidis[1], S. Beausiegle[1], R. Ohm[3], A. Aerts[3], I. Grigoriev[3], G. H. J. Kema[4], S. B. Goodwin[5], R. Hamelin[1]. 1) Forest Sciences, University of British Columbia, Vancouver, BC, Canada; 2) CFS Laurentian Forestry Centre, Succ. Sainte-Foy, Québec, Canada; 3) 3DOE Joint Genome Institute, Walnut Creek, California, USA, DOE, USA; 4) Plant Research International B.V., Wageningen, The Netherlands; 5) USDA-Agricultural Research Service, Purdue University, West Lafayette, Indiana, USA.

Two closely related, morphologically indistinct fungal pathogens of poplars, *Mycospharella populorum* and *M. populicola* are prevalent in North America. In natural stands, these two fungal species closely follow the distribution of their host, with *M. populorum* being found on Aiegeiros botanical section and *M. populicola* on the Tacamahaca section of poplars. Epidemiologically, *M. populorum* is considered to be more aggressive, as in addition to leaf-spots, it has the ability to infect woody tissue and cause cankers, an ability that *M. populicola* lacks. Moreover, introduction of hybrid plantations has added to *M. populorum* host range. Availability of genomes will allow us a window into understanding the genetic basis for these observed differences in epidemiology and host-specificity for these two pathogens. Historical observation of host-specificity was confirmed by comparative sequence analysis the estimated the divergence time between the two poplar pathogens to be ~6.4 Mya, which agrees with the divergence time estimates for the poplar botanical sections (6.8 - 7.8 Mya). Despite the remarkable macro-synteny exhibited between these two recently diverged pathogens, several genes specific to each pathogen were identified in genomic regions where synteny broke down. In addition to being candidates for the different physiological and epidemiological attributes, these species-specific genes could be utilized for diagnostic and monitoring assays. A consistent expansion of several pathogenicity-related gene families was observed in *M. populorum*, suggesting a role for gene-dosage in determining its ability to cause cankers. Preliminary enzyme assays showed significant differences in beta-glucosidase and xylanase activities between these two fungi.

333. Defining Open Chromatin Regions in *Coprinopsis cinerea* Oidia by FAIRE. Virginia K. Hench[1,2], Patricia J. Pukkila[1,2]. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

Changes in chromatin organization are principal regulatory mechanisms controlling multiple cellular processes including gene expression and meiotic crossover formation. Here we present FAIRE (formaldehye assisted isolation of regulatory elements) data that reveals regions of open chromatin in *Coprinopsis cinerea* oidia, the asexual spore stage of the *C. cinerea* life cycle. A standard FAIRE protocol was developed and optimized for oidia and used to enrich for nucleosome-free stretches of chromatin. FAIRE peaks were identified from single-end read whole genome sequence data using ZINBA (Zero-Inflated Negative Binomial Algorithm), which identified 7,276 peaks covering 6.3% of the genome. FAIRE peaks are predominantly intergenic with 78% of FAIRE domains overlapping noncoding sequence. The peak widths range from 98-1390 bps, with an average width of 310 bps. Nearly half or 47% of annotated genes (Broad version 3) contain a FAIRE peak in the proximal promoter region (defined as 500 bps immediately upstream of the gene start). Differential transcription has been characterized throughout the synchronous meiotic process in *C. cinerea* (Burns, C. et al., PLOS Genetics, vol 6, issue 9, 2010), but the extent to which nucleosome occupancy might contribute to gene regulation in this multicellular fungus was not known. We found that a minority of meiotic specific (MS; genes expressed in meiosis and not in vegetative tissue) genes had promoter FAIRE peaks (of 819 genes 37% had promoter FAIRE peaks). In contrast, 61% of genes significantly changing during meiosis (SCDM; 2,455 genes) had promoter FAIRE peaks. Out of 295 genes that were MS and SCDM, 38% had promoter FAIRE peaks in oidia. Genes with known meiotic function including *spo11*, *dmc1*, and *rec8* were amongst the MS/SCDM genes that did not have promoter FAIRE peaks in oidia. In summary, all examined meiotic gene sets included genes associated with and without FAIRE peaks in their promoter regions, indicating that complex gene regulation mechanisms contribute to differential, tissue-specific gene expression in *C. cinerea*. Supported by the U.S. Department of Energy Joint Genome Institute Community Sequencing Program. The work conducted by the U.S. DOE JGI is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

334. Ensembl Fungi - genome-scale data portal from fungal species. Uma Maheswari, Heldér Pedro, Mark McDowall, Daniel M. Staines, Paul Kersey. European Bioinformatics Institute (EMBL-EBI), Cambridge, United kingdom.

Ensembl Fungi (http://fungi.ensembl.org) is a portal offering access to genome-scale data from fungal species, using the Ensembl genome analysis system, through a common set of interfaces shared with non-fungal species also represented in the Ensembl system. These include a web-based genome browser, Perl and REST-ful APIs, a public MySQL server and a query-orientated data warehouse (BioMart). The current release (January 2013) provides access to 36 fungal genomes across 12 different taxonomic orders, including the model species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, (for which data are imported from the Saccharomyces Genome Database and PomBase respectively) but focuses mainly on plant pathogen species: genomic data from these is being integrated with information about infectious phenotypes (derived from PHI-base (http://www.phibase.org) on a per-gene basis, thro ugh a new targeted resource PhytoPath (http://www.phytopathdb.org).

Core data provided for all species includes genome sequence, sequence patterns, annotation of protein and non-coding genes and functional annotation imported from direct curation, UniProt and InterPro. Information about gene regulation, sequence variation, evolution and conservation is also integrated in the system. Protein alignments are used to reconstruct evolutionary trees and infer homology relationships, while pairwise alignments between DNA sequences are performed between closely related species. Genomic polymorphisms are presented in the context of the reference genome sequences of *Saccharomyces cerevisiae* and the phytopathogens *Gibberella zeae, Puccinia graminis* and *Fusarium oxysporum.*

Ensembl Fungi will continue to expand with the increase in genomic data , we seek to work with the communities actively generating and using data, and are participants in a growing range of collaborations involved in the annotation and analysis of genomes.

335. A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon. Alija Mujic, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Species of Rhizopogon are EM symbionts of trees in family Pinaceae and produce basidiospores within hypogeous false truffles that are dispersed by mycophagous mammals. All known members of R. subgenus Villosuli form obligate EM relationships with Pseudotsuga spp. (Douglas Fir) and are the only members of the genus known to possess this host association. R. vesiculosus, along with its cryptic sister species R. vinicolor, possess a sympatric distribution where sampled within the range of their host tree, P. menziesii. While the sporocarp and EM morphology of these fungi may be highly similar; they possess striking life history differences with R. vesiculosus producing larger vegetative genets and displaying greater population structure at both local and landscape scales. We have sequenced the genome of R. vesiculosus using dikaryotic tissue and a whole genome shotgun sequencing approach on the Illumina HiSeq platform. De novo assembly of the genome was performed using VELVET 1.19 and gene predictions were made using AUGUSTUS with Laccaria bicolor as a training model. The draft genome assembled to a total length of 46 Mb in 6700 contigs with an N50 of 26,783, a maximum contig size of 446,818 bp, and 12,604 predicted genes. Here we characterize the mating system of R. vesiculosus, which possesses both an A-locus encoding a heterodimer transcription factor, as well a B-locus encoding transmembrane pheromone receptors and pheromone precursor genes. We present comparisons of the mating system of R. vinicolor and its similarities to other members of Boletales (e.g., Serpula) and differences with Agaricales (e.g., Laccaria). Due to the dikaryotic nature of the genome sequence produced for R. vesiculosus, single nucleotide polymorphisms (SNPs) can be observed and used to characterize allelic variation. SNPs observed in protein coding regions of both MAT loci indicate that R. vesisculosus is likely heterothallic. We have also characterized heterozygosity across the whole genome in order to identify hypervariable regions. This genome will allow for comparative analysis of gene content, mating type system with other Basidiomycota and, ultimately, for population/species-level genomic studies within Rhizopogon.

336. Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. Robin A Ohm[1], Nicolas Feau[2], Bernard Henrissat[3], Conrad L Schoch[4], Benjamin A Horwitz[5], Rosie E Bradshaw[6], Lynda Ciuffetti[7], Richard C Hamelin[2,8], Gert HJ Kema[9], Christopher Lawrence[10], James A Scott[11], Joseph W Spatafora[7], B. Gillian Turgeon[12], Pierre JGM de Wit[13], Shaobin Zhong[14], Stephen B Goodwin[15], Igor V Grigoriev[1], Other members of the Dothideomycetes community. 1) United States Department of Energy (DOE) Joint Genome Institute (JGI), Walnut Creek, CA, United States of America; 2) Faculty of Forestry, Forest Sciences Centre, University of British Columbia, Vancouver, BC, Canada; 3) Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, CNRS, Marseille, France; 4) NIH/NLM/NCBI, Bethesda, MD, United States of America; 5) Department of Biology, Technion - IIT, Haifa, Israel; 6) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand; 7) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, United States of America; 8) Natural Resources Canada, Ste-Foy, QC, Canada; 9) Plant Research International, Wageningen, The Netherlands; 10) Virginia Bioinformatics Institute & Department of Biological Sciences, Blacksburg, VA, United States of America; 11) Division of Occupational & Environmental Health, Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; 12) Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY, United States of America; 13) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 14) Department of Plant Pathology, North Dakota State University, Fargo, ND, United States of America; 15) United States Department of Agriculture, Agricultural Research Service, Purdue University, West Lafayette, Indiana, United States of America.

The class *Dothideomycetes* is one of the largest groups of fungi with a high level of ecological diversity including many plant pathogens infecting a broad range of hosts. Here, we compare genome features of 18 members of this class, including 6 necrotrophs, 9 (hemi)biotrophs and 3 saprotrophs, to analyze genome structure, evolution, and the diverse strategies of pathogenesis. The *Dothideomycetes* most likely evolved from a common ancestor more than 280 million years ago. The 18 genome sequences differ dramatically in size due to variation in repetitive content, but show much less variation in number of (core) genes. Gene order appears to have been rearranged mostly within chromosomal boundaries by multiple inversions, in extant genomes frequently demarcated by adjacent simple repeats. Several *Dothideomycetes* contain one or more gene-poor, transposable element (TE)-rich putatively dispensable chromosomes of unknown function. The 18 *Dothideomycetes* offer an extensive catalogue of genes involved in cellulose degradation, proteolysis, secondary metabolism, and cysteine-rich small secreted proteins. Ancestors of the two major orders of plant pathogens in the *Dothideomycetes*, the *Capnodiales* and *Pleosporales*, may have had different modes of pathogenesis, with the former having fewer of these genes than the latter. Many of these genes are enriched in proximity to transposable elements, suggesting faster evolution because of the effects of repeat induced point (RIP) mutations. A syntenic block of genes, including oxidoreductases, is conserved in most *Dothideomycetes* and upregulated during infection in *L. maculans*, suggesting a possible function in response to oxidative stress.

337. Domains of meiotic DNA recombination and gene conversion in *Coprinopsis cinerea* (*Coprinus cinereus*). Patricia J. Pukkila[1], Wendy Schackwitz[2]. 1) Dept Biol, Univ North Carolina, Chapel Hill, NC, USA; 2) US DOE Joint Genome Institute, Walnut Creek, CA, USA.

We have shown previously that rates of meiotic recombination are highly non-uniform along the assembled chromosomes of *C. cinerea* (Stajich et al. PNAS 107: 11889-11894, 2010). That study revealed an over-representation of paralogous multicopy genes in regions with elevated levels of meiotic exchange. In addition, retrotransposon-related sequences were not found in large segments of the genome with low levels of meiotic exchange. However, the study was limited by the available markers, and only 31 Mb of the 36 Mb genome could be mapped. More recently, we have resequenced 45 meiotic segregants and 4 complete tetrads. We developed a simple script to detect crossover and gene conversion events involving over 75,000 SNPs spanning 35 Mb. The data were analyzed using MSTmap (Wu et al. PLoS Genetics 4: e1000212, 2008). The new dataset revealed sub-telomeric recombination hotspots at every chromosome end, and 36% of the crossovers were associated with uninterrupted tracts of gene conversion. The conversion tracts (2-8 SNPs) were quite short (8-219 nt), and the median distance between the flanking SNP markers was also small (500 nt). Since these subtelomeric hotspots correspond to sites of synaptic initiation in *C. cinerea* (Holm et al. Carlberg Res. Commun. 46: 305-346, 1981), these data may contribute to our understanding of how homologous chromosome pairing and synapsis are coordinated with meiotic recombination. Supported by the U.S. Department of Energy Joint Genome Institute Community Sequencing Program. The work conducted by the U.S. DOE JGI is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

338. FungiDB: An integrated functional genomics database for fungi. Raghuraman Ramamurthy[1], Edward Liaw[1], Sucheta Tripathy[7], John Brestelli[2,3], Sufen Hu[3], Wei Li[3], Omar Harb[3,4], Brian Brunk[3,4], Steve Fischer[2,3], Deborah Pinney[2,3], Jessica Kissinger[5,6], Brett Tyler[8], David Roos[3,4], Jason Stajich[1]. 1) Plant pathology and Microbiology, University of California, Riverside, Riverside, CA; 2) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Penn Center for Bioinformatics, University of Pennsylvania, Philadelphia, PA; 4) Department of Biology, University of Pennsylvania, Philadelphia, PA; 5) Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA; 6) Department of Genetics and Institute of Bioinformatics, University of Georgia, Athens, GA; 7) Virginia Bioinformatics Institute, Virginia Tech University, Blacksburg, VA; 8) Center for Genome

Research and Biocomputing, Oregon State University, Corvallis, OR.

FungiDB (http://FungiDB.org) is a functional genomic database and website tool for fungal genomes to enable data mining and analyses of the pan-fungal genomic resources. The resource was developed in partnership with the Eukaryotic Pathogen Bioinformatic Resource Center (http://EuPathDB.org). Using the same infrastructure and user interface as EuPathDB, FungiDB allows for sophisticated and integrated searches to be performed over an intuitive graphical system. The new 2.2 release contains sequence and annotation for over 50 species spanning the Ascomycota, Basidiomycota, Zygomycota, and Chytrid fungi; including pathogenic species from the *Cryptococcus*, *Histoplasma*, and *Coccidiodes* genera. Six Oomycete genomes from *Phytophthora* and *Pythium* species and RNA-Seq data are also included in the release of the system. Data from *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus nidulans*, and *Neurospora crassa* represent the latest annotation releases of these genomes.

Functional genomics data is available for querying including gene expression data from microarray, RNA-Seq, and expressed sequence tags; yeast two hybrid interaction data; and gene ontology from curated and automated sources. New features in the 2.2 release include population genomics data of SNPs for several ascomycetes including *A. fumigatus*. A user interface to the precomputed orthology and paralogy of complete gene sets from the supported fungal genomes along with key metazoan, plant, microbial eukaryotes, and bacteria enable phylogenetic profiling across the tree of life. The data-mining interface also permits the ability to make inferences using functional data in one species transformed by orthology into another species, providing a powerful resource for in silico experimentation. Query strategies from the system can be saved and shared as web links to enable reproducible results. FungiDB is supported by the Burroughs Wellcome Fund and the Alfred P. Sloan Foundation.

339. Letters from the front: The *Microbotryum violaceum* genome and transcriptome project. Su San Toh[1], Jared Andrews[1], Sébastien Duplessis[2], David Treves[3], Christina Cuomo[4], David Schultz[1], Michael Perlin[1]. 1) University of Louisville, Louisville, KY, USA; 2) Centre INRA de Nancy, Champenoux, France; 3) Indiana University Southeast, New Albany, Indiana, USA; 4) Broad Institute, Cambridge, Massachusetts, USA.

Microbotryum violaceum is a fungal species complex that includes related smut species primarily infecting members of the Caryophyllaceae (pinks). Individual species of this group are limited to successful infection and reproduction on a specific host species. We have produced a draft sequence at 18x coverage for a haploid strain derived from meiosis of teliospores isolated from the host *Silene latifolia*. The draft sequence is currently in the process of annotation and is publicly available through a website at the Broad Institute. Using Illumina Next Gen sequencing, we are generating deep transcriptome information about a variety of stages in the lifecycle of the fungus, with particular emphasis on the late stages of infection, where teliosporogenesis occurs. Through the analysis we have performed so far, we were able to identify a suite of secreted proteins (SPs) that are potentially involved in host-pathogen interactions. Some of these include plant cell degradation enzymes like pectinesterase, laccase, subtilase and glycoside hydrolase. Moreover, some of these SPs are small, unique and cysteine-rich proteins, that might be involved in pathogenicity. Finally, since no reliable transformation system has been adapted for this fungus and, as a consequence, no targeted gene disruption has been demonstrated, we are developing constructs that rely on the newly completed genome to devise new strategies to allow such functional analyses in the future.

340. The *Aspergillus* and *Candida* Genome Databases: Recent Developments and Future Plans. Martha B. Arnaud[1], Gustavo C. Cerqueira[2], Diane O. Inglis[1], Marek S. Skrzypek[1], Jonathan Binkley[1], Clinton Howarth[2], Prachi Shah[1], Farrell Wymore[1], Gail Binkley[1], Stuart R. Miyasato[1], Matt Simison[1], Gavin Sherlock[1], Jennifer Russo Wortman[2]. 1) Dept. of Genetics, Stanford University School of Medicine, Stanford, CA; 2) Broad Institute, Cambridge, MA.

The *Aspergillus* and *Candida* Genome Databases (AspGD, http://www.aspgd.org and CGD, http://www.candidagenome.org/) are freely available, web-based resources for researchers studying the molecular biology of these fungi. The interfaces of both web sites and databases now provide streamlined, ortholog-based navigation of the genomic and functional annotation for multiple species concurrently. We have completed manual curation of the published literature about multiple *Candida* and *Aspergillus* species. As part of our community-oriented mission, we also provide resources to foster interaction and dissemination of community information, tools, and data, including collecting, archiving, and providing large-scale datasets for download. AspGD also offers a full-featured genomics viewer to facilitate comparative genomics analysis. We have added new servers to improve web site performance and page loading speeds. Areas of future expansion include incorporation and curation of additional species, as well as improvements to the reference genome sequences and gene sets, utilizing high-throughput sequence to correct errors in sequence and gene structure, and display of additional regulatory elements and gene products, including alternate splice forms. We also plan to develop and incorporate improved tools for query, display and analysis of data, especially large-scale and comparative data such as gene synteny and the evolution of genes and gene substructure (e.g., intron gain and loss). We welcome, encourage, and appreciate your questions, feedback or suggestions. AspGD and CGD curators can be reached at aspergillus-curator@lists.stanford.edu and candida-curator@lists.stanford.edu, respectively. AspGD is funded by grant R01 AI077599 from the National Institute of Allergy and Infectious Diseases, and CGD is funded by R01 DE015873 from the National Institute of Dental and Craniofacial Research at the US National Institutes of Health.

341. The *Trichoderma reesei* polyketide synthase gene *pks1* is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness. Lea Atanasova[1], Benjamin P. Knox[2], Christian P. Kubicek[1], Scott E. Baker[2], Irina S. Druzhinina[1]. 1) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; 2) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, Richland, WA, USA.

The economically important genus *Trichoderma* (Hypocreales, Ascomycota, Dikarya) is well known for its mycotrophic lifestyle and for the broad range of biotrophic interactions with plants and animals. Moreover it contains several cosmopolitan species characterized by their outstanding environmental opportunism. These properties have given rise to the use of several species in agriculture as biopesticides and biofertilizers while *T. reesei* is applied for production of bioenergy-related enzymes. The molecular basis of the opportunistic success of *Trichoderma* is not yet well understood. While there is some evidence for a role of secreted enzymes and proteins, less is known about a possible role of secondary metabolites. Recently it was predicted that the PKS encoding gene *pks1* from *T. reesei* and its orthologues are most likely responsible for the characteristic yellow-green pigmentation of conidia. To reveal the full function of the gene we deleted it from the wild-type strain QM 6a what resulted in complete loss of the green coloration of conidia. The ecophysiological profiling of *Dpks1* showed that the gene is also involved in multiple functions at different stages of the *T. reesei* life cycle. Testing the antagonistic antifungal potential of the *T. reesei Dpks1* mutant against several host/prey fungi suggested that the loss of *pks1* reduced the ability to combat them by means of both mechanisms: the pre-contact inhibition and direct overgrowth. However the overall analysis of mycoparasitic interactions suggests that the gene is most likely involved in protection against other fungi rather than in attacking them. Interestingly, we noticed the increased production of volatile compounds by the *Dpks1* strains. The phenotype microarrays showed that PKS1 encoding gene restricts *T. reesei* from conidiation on a number of the best utilized carbon sources but does not influence the sexual development except the alteration of stromata pigmentation. The data for transcriptional response of genes putatively involved in above mentioned processes will be presented.

342. Functional Analysis of Genes in Regions of Introgression in Coccidioides. Bridget M. Barker. Immunology & Infectious Diseases, Montana State Univ, Bozeman, MT.

Coccidioides immitis and C. posadasii are dimorphic fungi endemic to the Americas. Genomic analysis of sequenced strains of C. posadasii and C. immitis reveals insights into the population biology of these organisms. There is strong evidence for hybridization and introgression, such that for many of the C. immitis strains, there are several regions that have a closer match to C. posadasii, but few regions within C. posadasii matching C. immitis. Multiple hybridization regions were located in several genomes analyzed, and at least one region containing ten genes exhibits a pattern consistent with introgression in C. immitis. This conserved region was further evaluated in a larger collection of isolates. Approximately half of the C. immitis isolates contain the C. posadasii fragment, and the majority of those are from the southern California and Mexico populations. The region of introgression represents a unique opportunity to functionally assess genes that are likely to be relevant for species-specific virulence and adaptation to mammalian hosts or the environment. This region has a shared recombination point flanking a metalloproteinase, Mep4; genes that are highly expressed in the parasitic phase; and genes of unknown function. Importantly, evolutionary selection has preserved this region in multiple strains of C. immitis further emphasizing the possible role in virulence of these genes. Variation among strains for virulence in murine models of coccidioidomycosis has been observed, but has not been tested in the context of the newly discovered species or with a targeted underlying genetic mechanism hypothesis to test. Gene deletion mutants are being generated for three genes in the conserved introgression region to determine effects on in vitro growth and morphological change under host relevant conditions.

343. Classification and accurate functional prediction of carbohydrate-active enzymes by recognition of short, conserved peptide motifs. Peter K. Busk, Lene Lange. Biotechnology and Chemistry, Aalborg University, AAU Cph, Copenhagen, Copenhagen, Denmark.

Functional prediction of carbohydrate-active enzymes is difficult due to low sequence identity hampering recognition of the functional relationship. However, similar enzymes often share a few short motifs, e.g., around the active site even when the overall sequences are very different. To exploit this notion for functional prediction of carbohydrate-active enzymes we developed a simple algorithm, Peptide Pattern Recognition (PPR) that can divide proteins into groups of sequences that share a set of short conserved sequences. When this method was used on 118 functionally characterized GH5 proteins with 9 % average pairwise identity and representing four enzymatic functions, 97 % of the GH5 proteins were sorted into groups correlating with their enzymatic activity. Furthermore, we analyzed 8138 GH13 proteins including 204 experimentally characterized enzymes with 28 different functions. There was a 91 % correlation between group and enzyme activity. These results indicate that the function of carbohydrate-active enzymes can be predicted with high precision by finding short conserved motifs in their sequences. The GH61 family is important for fungal biomass conversion but only few GH61s have been functionally characterized. Interestingly, PPR divided 743 GH61 proteins into 16 subfamilies useful for targeted investigation of the function of these proteins, and pinpointed three conserved motifs with putative importance for enzyme activity. The conserved sequences were useful for discovery and cloning of new, subfamily-specific GH61 proteins from 14 different fungi. In conclusion, identification of conserved sequence motifs is a new approach to sequence analysis that can predict carbohydrate-active enzyme functions with high precision. Furthermore, these motifs can be used to mine genomes and more complex data such as metagenomes and -transcriptomes for genes encoding proteins with specific, enzymatic activity.

344. The mechanism of introner-like element multiplication in fungi. Ate van der Burgt[1], Edouard Severing[2], Valeria Ochoa Tufiño[1], Pierre de Wit[1], Jérôme Collemare[1]. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Laboratory of Bioinformatics, Wageningen University, 6708PB Wageningen, The Netherlands.

The recent discovery of introner-like elements (ILEs) in six fungal species shed new light on the origin of regular spliceosomal introns (RSIs). ILEs are novel spliceosomal introns that are found in hundreds of near-identical copies in unrelated genes. They account for the vast majority of intron gains in these species and are not associated with intron losses. Remarkably, ILEs are longer than RSIs and harbor predicted stable secondary structures. However, they are prone to quickly degenerate in sequence and length to become undistinguishable from RSIs, suggesting that ILEs are predecessors of most RSIs. Further analyses are being performed in order to understand the multiplication mechanism of ILEs, which is hypothesized to resemble the retro-homing mechanism of self-splicing group II introns. The dynamics of ILE's secondary structures could be predicted and two conserved motifs were identified in almost all fungal ILEs, which might play an important role in direct insertion into DNA. We also have developed a genetic screen in yeast in order to capture and characterize ILE insertion events. These ongoing studies should provide hints about the mechanism of ILE multiplication, i.e. how new spliceosomal introns are gained in fungi.

345. Fungi use prion folds for signal transduction processes involving STAND proteins. Asen Daskalov, Khalid Salamat, Sven J. Saupe. CNRS, IBGC UMR5095, BORDEAUX, AQUITAINE, France.

Prions are proteins embedding genetic information into their structural state. Generally, those proteins exist in a soluble state and sporadically as infectious amyloid aggregates. Podospora anserina's [Het-s] is one of the best characterized fungal prions with a remarkably high prevalence in wild populations. [Het-s] functions in vegetative incompatibility - a biological process occurring during anastomosis between two genetically incompatible strains. The HET-s protein exists in a soluble state - [Het-s*] - or can switch to an aggregated amyloid state - [Het-s] - the prion form. When an [Het-s] prion infected strain fuses with a strain expressing the alternative allelic variant of the het-s locus - het-S - a cell death reaction of the heterokaryon occurs. Recent studies shed light on the mechanism of [Het-s]/HET-S incompatibility reaction. Differing by 13 amino acids both proteins shares a two domain architecture; a globular N-terminal domain called HeLo and a C-terminal Prion Forming Domain (PFD). The latter is able to adopt a b-sheet rich conformation with a specific b-solenoid fold. It has been demonstrated that in presence of [Het-s] amyloid fibers HET-S turns into a pore-forming toxin: transconformation of the HET-S PFD by [Het-s] fibers triggers the refolding of the HET-S HeLo domain, inducing the cell death reaction. In an attempt to better characterize the conserved features of the [Het-s] b-solenoid fold and identify new distant homologues of HET-S/s, we have generated a minimal consensus sequence motif of it. Surprisingly, the second best hit in a BLASTp search is in the N-terminal region (3-23) of the product encoded by nwd2, the immediately adjacent gene to het-S. NWD2 is a STAND protein. STAND proteins form signal transducing hubs through oligomerization upon ligand recognition. That in mind and several bioinformatics observations led us to propose that HET-S and NWD2 are functional partners in various filamentous fungal species using the amyloid fold in a signal transducing pathway. We will present experimental evidence that NWD2 is able to trigger HET-S toxicity in much the same way as [Het-s] does. Further in silico analysis identify a number of these STAND/prion-like gene pairs and suggest that signal transduction through an amyloidal prion-like fold is a general widespread mechanism in fungi.

346. RNA silencing in poplar anthracnose fungus *Colletotrichum gloeosporioides*. Simeng Li, Yonglin Wang, Chengming Tian. The Academy of Forestry, Beijing Forestry University, Beijing, China.

Poplar anthracnose is one of the most destructive diseases on *Poplus sp*, whose causal agent is *Colletotrichum gloeosporioides*. Although the fungus is a

broad-host range plant pathogen, only dozens of genes involved in pathogenesis have been identified and characterized. In order to establish a high-throughput platform,this study delivered the double-stranded RNA(dsRNA) expression cassette into protoplasts to trigger silencing for functional genomics research in *C. gloeosporioides*.A new silencing vector pSD-SUR1 based on RNA-silencing vector (pSD1) with a convergent dual promoter was introduced. In this silencing system, the target gene was proposed to be transcribed as a chimeric RNA which activates the system. As an indicator of gene silencing, GFP fluorescence is used to evaluate efficiency of this silencing system. The fluorescence observation showed GFP fluorescence significantly decreased in some of the silenced strains, comparing with the recipient strain. The GFP mRNA transcript levels in the strains were analyzed using quantitative RT-PCR. The results showed that the reduction range of controls in *gfp* expression was from 30% to 80%,suggesting an effective gene silencing system and a feasible approach to generate detectable phenotypes in *C. gloeosporioides*. In addition, some genes encoding signal transduction pathways and transcriptional factor were inserted respectively into the vector pSD-SUR1 and to be silenced. In conclusion, RNA silencing system opens up new opportunity for exploring gene function in the fungus *C. gloeosporioides*.

347. Comparative Genomics of L and S Morphotypes of *Aspergillus flavus*. Mana Ohkura, Peter Cotty, Marc Orbach. Division of Plant Pathology, University of AZ, Tucson, AZ.

Aspergillus flavus is a widely distributed facultative pathogen of plants and animals and the most common causal agent of crop contamination with aflatoxins. Isolates of *A. flavus* vary widely in aflatoxin producing ability, ranging from atoxigenic to being capable of producing many mg/g. Variability in aflatoxin production makes specific attribution of etiology very complex. *Aspergillus flavus* exists in two morphotypes the large (L) and small (S) sclerotial producing strains. The S strains have consistent high aflatoxin-producing ability while the L strains vary greatly in toxin production with atoxigenic strains commonly found. Some atoxigenic strains are active ingredients in biocontrol products used commercially to prevent contamination. We are applying comparative genomics to L and S strains in an attempt to reveal clues to potential differential adaptations associated with the variation in aflatoxin-production between these morphotypes. In addition to aflatoxin producing potential, several characteristics diverge between the morphotypes including sclerotial morphology as well as spore and hydrolase production and prevalence. We hypothesize there are genomic differences between the L and S morphotypes that reflect their divergent evolution leading to differential adaptation. To evaluate this, we have sequenced the genomes of three L morphotype and three S morphotype isolates from agricultural fields in Arizona belonging to 6 different vegetative compatibility groups. L and S strain isolates from across Arizona were selected. Strains were sequenced to ~40-45 X coverage on the Illumina HiSeq 2000 platform. The genomes were assembled *de novo* using VelvetOptimiser and gaps were filled using GapFiller. Preliminary assessment of the assemblies indicate there is ~90% syntenic coverage with the published genome of *A. oryzae* RIB40, a close relative of A. flavus. The genomes were annotated transitively with RATT using the genome of *A. oryzae* RIB40 as a reference. Comparisons of genome statistics, secondary metabolite clusters, and morphotype specific genes will be presented.

348. Searching for Functional Mobile Elements in *Coprinopsis cinerea*. Kendra Boyd, Madhura Chitnavis, Marilee A. Ramesh. Dept Biol, Roanoke College, Salem, VA.

The genome of *Coprinopsis cinerea* contains both Class I and Class II repetitive elements, making up about 2.5% of the total genomic DNA. While bioinformatics techniques were used to identify and classify these elements based on sequence similarity, it is uncertain whether any of the elements are functional. Although the nature of repetitive elements is to expand their numbers within the genome, the genome acts to suppress activity through mutation and methylation. Detailed analysis to survey functionality and expression was conducted on two families of repetitive elements, the Gypsy-like retrotransposons (Class I) and the hAT Transposons (Class II). The potential for functionality was determined based on size, structure and flanking repeat sequence integrity. Evidence for expression was determined based on reviewing EST, SAGE and methylation data for these elements. Of the 31 largest gypsy elements analyzed, all appear to be inactive. However, one of the nine hAT elements appears to be structurally intact and shows evidence of expression. The dimerization domain of this element is being studied as a potential region to assay for activity.

349. Genome-wide analysis of small RNA machineries in fungal kingdom. Jiayao Wu[1], Jaeyoung Choi[2], Fred O. Asiegbu[1], Jari P.T. Valkonen[1], Yong-Hwan Lee[1,2]. 1) Forest Science, Helsinki University, Helsinki, Finland; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea.

RNA interference (RNAi) is a phenomenon widely conserved in eukaryotes to regulate gene expression through diverse pathways at transcriptional (TGS) or post-transcriptional level (PTGS). In fungi, the RNAi pathways are found with three major functions: genomeic defence, heterochromatin formation, and gene regulation. The mechanisms of RNAi in fungi seem to be unique and highly differentiated from plant and animal kingdoms, although the core mechanisms are relatively similar. We identified 3 key genes such as Argonaute, Dicer and RNA-dependent RNA Polymerase (RdRP) in the pathway from 143 fungal and 66 other genomes. They were found in most genomes with very different gene numbers, while some of fungal genomes appear to be lack of all the components indicating the absent of the whole pathways. In general, fungi have the same domains in Argonauts with plant and animal, but longer in the length and less in the number. Compared to plant and animal, fungi have more Dicers, but they do not contain PAZ domain, which is essential for RNAi in plant and animal. Phylogentic analysis indicates that most fungal Argonuates belong to AGO-like subfamily. However, fungal Dicers could to be divided into two subfamilies; one is closely related to plant and animal Dicers and the other only exists within fungal kingdom. Further analysis using codonW shows RNAi proteins are evolved into different subfamilies under natural selection not due to random mutation. Taken together fungi RNAi pathway is likely to be much complex than we expected with multiple functions in diverse regulatory pathways. All information on proteins analyzed is archived in Fungal Small RNA Machinery Database (http://funrna.riceblast.snu.ac.kr/).

350. Comparative Analysis of *Malassezia* Mating Loci. Jun Xu[1], Wenjun Li[2], Anastasia Giotia[3], Björn Nystedt[4], Anna Averettec[2], Charles Saunders[1], Thomas Dawson[1], Joseph Heitman[2], Annika Scheyniuse[5]. 1) Procter & Gamble Co., Mason Business Center, USA; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, USA; 3) Science for Life Laboratory, Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden; 4) Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; 5) Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden.

Malassezia fungi are naturally found on the skin surfaces of many animals and are associated with skin disorders such as dandruff and atopic dermatitis/eczema. Whole genome sequence analysis showed that *M. globosa* has a bipolar mating type with two *MAT* alleles encoding the homeodomain (HD) and pheromone/receptor (P/R) loci separated by 167 kb of intervening sequence. We compared the *M. globosa* mating locus with the newly sequenced *M. sympodialis* genome. Our analysis showed that the *M. sympodialis* MAT region has extensive well-conserved synteny with *M. globosa* and the HD and P/R mating subloci are physically linked with a 141 kb separating the two. Interestingly, *MAT* sequences derived from a population of *M. sympodialis* isolates suggests that *M. sympodialis* does not fit a traditional bipolar or tetrapolar system. Instead, it is more similar to a pseudo-bipolar model previously reported for *Sporidiobolus salmonicolor* in which the HD and P/R genes are physically linked similar to bipolar mating type configurations.

This is in contrast to bipolar systems where recombination can still occur, giving rise to more mating types similar to tetrapolar mating systems. We also provide some initial comparative analysis of the *MAT* region of *M. restricta*.

Education and Professional Development

351. The internet effectiveness for gaining students enrolled at college of education The scientific facts and concepts about Biofuel issue according to the Responsibility Spiral model. Khlood S AlSheikh. Science Education, K.A.U, Jeddah, Saudi Arabia.

This study was conducted to ascertain the effectiveness of the internet gaining students enrolled at college of education the scientific facts, concepts and disagreement about Biofuel issue according to the Responsibility Spiral model. The study used a quasi-experimental design. The study sample consisted of 76 students divided into two groups. The experimental group consisted of 37 students, whereas the control group consisted of 39 students. The study used materials and activities according to the Responsibility Spiral model. Each stages of the waks (1992) model consisted of objectives, content, teaching and learning activities and evaluation tools. The results showed statistically significant differences between the mean score of the experimental group which gained the scientific facts and concepts about Biofuel issue according to the Responsibility Spiral model and the mean score of the control group. The result showed that there is an opposite relationship between the model stages and t-test that if the model expands the value of t-test decreased. Based on the results, the researcher recommends that the internet is not enough tools for advanced stages of the Responsibility Spiral model.

352. ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis. Gita Bangera[1], Andrea Gargas[2]. 1) Bellevue College, Bellevue, WA, USA; 2) Symbiology LLC, Middleton, WI, USA.

ComGen (Community College Genomics Research Initiative) teaches students the skills of self-directed learning, critical thinking, and analysis. Community college students in this program receive a mini-graduate school experience, following a single requisite course in cell biology. Students work on original research projects, learn to troubleshoot their experiments, organize lab meetings and student journal clubs, and network within the scientific community. In one research track students work with DNA from described fungal collections, learning DNA-based techniques including PCR amplification, DNA sequencing and sequence analysis. Student-gathered sequence information is used to advance identification and phylogenetic results for these collections. With NSF Award DUE #1225857 ComGen (C-ARE): Dissemination, Enrichment and Expansion Project the project will be expanding to community college partners throughout the Seattle/Tacoma region of Washington State.

353. Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread. Virginia K. Hench[1,2], Patricia J. Pukkila[1,2]. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

The HHMI-Future Scientists and Clinicians (HHMI-FSC) fellowship is 1 of 3 components of the HHMI Science Learning Communities program at UNC Chapel Hill. The HHMI-FSC program was designed to foster an intellectual community that empowers high-ability students from low-income backgrounds to engage in biomedical research for 2 summers. Each year, 12 new fellows are matched with mentors in labs spanning a range of biomedical areas. They work fulltime in labs on their own research project and meet weekly as a group to engage in interactive programming that targets skills critical for success in science beyond the bench. One area of emphasis has been the process of inquiry itself. The goal is for students to transition from being a pair of hands executing protocols to active learners invested in their own projects and able to speak with authority about why experiments are performed in particular ways and what conclusions can be drawn from data generated. This starts with coaching students to state the questions that they are trying to answer and think through whether an experimental setup is consistent with what they say they are trying to find out. Assignments and feedback are designed to reinforce this principle. One of the most satisfying aspects of doing science is getting to follow one's own instinctive curiosities and develop the methodologies needed to navigate new terrains. Undergraduates are usually still trying to define their own specific curiosities. Pushing students to describe what they are curious and passionate about is one feasible strategy that can help students identify pursuits that fit their interests and talents. Another successful strategy has been to require returning second year fellows to share science learning experiences via 15-30 minute long talks for their peers. Some took the opportunity to become more immersed in their lab's focus, while others branched into questions like what motivates scientists to work in foreign countries and what has genomic anthropology told us about human evolution. Project aims were developed through conversations between the fellow and instructor. The one constraint was for fellows to organize their presentations around questions. Feedback indicated that presenters benefited from having to give presentations and others enjoyed learning about a broader array of topics.

Gene Regulation

354. YAB- An *Agrobacterium*-based vector system for direct cloning of eukaryotic gene constructs via yeast recombination. M Alejandra Mandel, Marc J Orbach. School of Plant Sciences and The Bio5 Institute, University of Arizona, Tucson, AZ.

Agrobacterium tumefaciens-mediated transformation (ATMT) has become the preferred method to introduce modified genes in many fungal systems. Therefore, there is a need for a simple and efficient method to create gene constructs in an ATMT vector. Generation of ATMT constructs usually requires PCR-amplification of DNA fragments and multiple cloning steps into binary *Agrobacterium* vectors. Advances have been made with attB/attP-based systems (e.g. pTroya, Gene-blast, DelsGate) that shorten the number of steps required for generating plasmid constructs, however these constructs require a final sub-cloning step into an *Agrobacterium* vector. One of these systems, OSCAR, combines PCR and attB/attP-based technology in an *Agrobacterium* platform. All of these methods require the use of commercial enzymes and usually involve one or more PCR amplification steps. There are also plasmid vectors that use yeast transformation-associated recombination (TAR) which do not require the use of enzymes for cloning, but can only be used to transform fungi via electroporation, protoplast transformation or biolistics. We present here YAB (for Yeast-*Agrobacterium*-Bacteria), a vector that combines the homologous recombination properties of yeast in an *Agrobacterium* vector backbone that can be used to create any kind of gene construct (e.g. gene deletion mutants, fluorescence-tagged genes, overexpression, RNAi) to directly transform fungi in one step. DNA fragments are cloned into YAB via recombination between 22-nucleotide compatible ends generated by PCR, or by using short oligomers that "bridge" the DNA fragment with the vector, thus avoiding the need for amplification steps. YAB-Hph has a 1.4-kb Hygromycin phosphotransferase cassette flanked by the left and right borders of the Ti plasmid. This vector was used to generate whole-gene deletion mutants by TAR in one step, that were directly introduced into fungi.

355. Removal of C4-methyl Sterol Accumulation in a SREBP-null Mutant of *Aspergillus fumigatus* Restores Hypoxia Growth. Sara J. Blosser[1], Brittney

Hendrickson[1], Nora Grahl[2], Dawoon Chung[2], Bridget Barker[1], Robert A. Cramer[2]. 1) Immunology & Infectious Disease, Montana State University, Bozeman, MT; 2) Microbiology & Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH.

The human pathogen *Aspergillus fumigatus* adapts to stress encountered in the mammalian host based on evolutionary mechanisms derived from its ecological niche as a composter or saprobe. SrbA, a member of the helix-loop-helix family of transcription factors, plays a significant role in *A. fumigatus* hypoxia adaptation, antifungal drug responses, and virulence. SrbA is a direct transcriptional regulator of several key enzymes in the ergosterol biosynthesis pathway, which has been verified by *in vivo* ChIP-SEQ analyses. The sterol intermediate profile of D*srbA* revealed a significant accumulation of C4-methyl sterols, which correlates with the loss of *erg25* (C4-sterol methyl oxidase) mRNA abundance in the SrbA-null mutant. We hypothesized that this C4-methyl sterol accumulation may contribute to the significant phenotypes observed in D*srbA*. We have characterized the two genes predicted to encode C4-methyl sterol oxidases (Erg25) in *A. fumigatus*. Genetic deletion of both *erg25* genes, A and B, is lethal in *A. fumigatus*, while single genetic deletions of the respective genes are viable. Although loss of both *erg25A* and *erg25B* resulted in accumulation of C4-methyl sterols, D*erg25A* accumulated far more C4-methyl sterol intermediates than D*erg25B*, suggesting that Erg25A is the predominant C4-sterol methyl oxidase in *A. fumigatus*. No dramatic *in vitro* or *in vivo* phenotypes under various stress conditions were observed in D*erg25A* or D*erg25B* mutants; however, a moderate reduction in hypoxia growth was observed in D*erg25A*. Generation of a strain that constitutively expresses *erg25A* in the D*srbA* background biochemically relieved a majority of the C4-methyl sterol buildup in D*srbA*. Significantly, restoration of *erg25A* mRNA levels in D*srbA* with a promoter replacement fully restored the *in vitro* hypoxia growth defect of D*srbA*. These results indicate that *erg25* transcriptional regulation by SrbA and management of C4-methyl sterol intermediate accumulation is highly important for hypoxia stress adaptation in *A. fumigatus*. Future studies will explore the impact of reducing C4-methyl sterol levels in D*srbA* on *A. fumigatus* virulence.

356. VeA Regulates Conidiation, Gliotoxin Production and Protease Activity in the Opportunistic Human Pathogen *Aspergillus fumigatus*. Sourabh Dhingra[1], David Andes[2], Ana M. Calvo[1]. 1) Biological Sciences, Northern Illinois University, DeKalb, IL; 2) Medical Mycology and immunology, University of Wisconsin-Madison, Madison, WI.

Aspergillus fumigatus is the causative organism of invasive aspergillosis. Our study shows that normal levels of *veA* expression are necessary for wild-type morphological differentiation in this medically important fungus. Specifically, deletion or overexpression of *veA* reduce conidiation. Parallely, *brlA* expression was also affected by alterations in *veA* transcription levels. In addition, our studies revealed that *veA* regulates gliotoxin production in *A. fumigatus*. Gliotoxin production was decreased in the deletion *veA* and over-expression *veA* strains, where *gliZ* and *gliP* expression was altered. Interestingly, *veA* also controls hydrolytic enzyme activity in this human pathogen. Deletion of *veA* resulted in a reduction of protease activity; this is the first report of a *veA* homolog with a role in controlling fungal hydrolytic activity. Although *veA* affects several cellular processes in *A. fumigatus*, pathogenicity studies in a neutropenic mouse infection model indicated that *veA* is dispensable for virulence. More research will be conducted to study the effect of veA in non-neutropenic mice.

357. HapXcess and C-terminal truncation impairs *Aspergillus fumigatus*' iron homeostasis. Fabio Gsaller[1], Veronika Klammer[1], Beatrix E. Lechner[1], Peter Hortschansky[2], Axel A. Brakhage[2], Ernst R. Werner[3], Hubertus Haas[1]. 1) Division of Molecular Biology, Medical University of Innsbruck, Austria; 2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 3) Division of Biological Chemistry, Medical University of Innsbruck, Austria.

The maintenance of iron homeostasis is indispensable as iron is essential for various cellular processes but can be toxic at iron excess. In *A. fumigatus* the bZIP-like transcription factor HapX is important for adaption to iron starvation and consequently virulence due to its role in repression of iron consuming pathways (i.e. heme biosynthesis, TCA cycle, respiration) and activation of iron uptake (i.e. siderophore biosynthesis and uptake, reductive iron assimilation). In this study we demonstrate that conditional *hapX* overexpression using the xylose-inducible *xylP* promoter leads to repression of genes involved in iron consumption (i.e. heme biosynthetic *hemA* and leucine-biosynthetic *leuA*) and activation of iron acquisition-related genes (i.e. siderophore-biosynthetic *sidG* and siderophore transporter-encoding *mirB*) within one hour of induction. In agreement, elevated *hapX* expression decreased the cellular accumulation of protoporphyrin IX, the iron-free precursor of heme, and increased production of the extracellular siderophore TAFC. HapX-truncation studies revealed that the C-terminal 93 amino acid residues are essential for its activating as well as repressing functions. HapX N-terminally tagged with Venus green fluorescent protein localized to the nucleus during iron starvation but was undetectable after an one hour-shift to iron sufficiency. These data demonstrate tight iron-regulation of *hapX* expression at the protein level as previously shown at the transcript level. Consistently, HapX-deficiency is detrimental only during iron limitation. Two *hapX* copies and in particular *xylP* promoter-mediated overexpression of *hapX* caused growth defects independent of the iron availability, which underscores the importance of a precisely regulated HapX level. This work was supported by the Austrian Science Foundation grant FWF P21643-B11 to HH.

358. The CCAAT-Binding-Complex mediates Iron Regulation in *Aspergillus fumigatus*. Hubertus Haas[1], Christoph Joechl[1], Thorsten Heinekamp[2], Ilse D. Jacobsen[2], Markus Schrettl[1], Axel A. Brakhage[2], Lukas Schafferer[1]. 1) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Austria; 2) Department for Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany.

Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved fine-tuned mechanisms for uptake and storage of iron, to sustain iron homeostasis. In the opportunistic fungal pathogen *Aspergillus fumigatus*, the bZIP-type transcription factor HapX mediates adaption to iron starvation by activating siderophore biosynthesis and repressing iron-dependent pathways. HapX-deficiency attenuates the virulence of *A. fumigatus* underlining the importance of adaptation to iron starvation in pathogenicity. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes and believed to co-regulate up to 30% of all genes. Here, we characterized the role of the CBC in iron regulation of *A. fumigatus* by analysis of the phenotypic consequences of genetic inactivation of the CBC subunit HapC. HapC-deficiency was deleterious during both iron starvation as well as iron sufficiency, demonstrating iron-independent regulatory functions of the CBC. In contrast, HapX is important during iron starvation only. As shown previously for HapX-deficiency, HapC-deficiency derepressed genes involved in iron-consuming pathways during iron starvation but decreased siderophore metabolism at transcriptional and metabolic levels. Inhibition of reductive iron assimilation by ferrous iron chelation blocked colony formation of both HapC-deficient and HapX-deficient conidia. Moreover, inactivation of HapC was epistatic to HapX-deficiency. Taken together, these data indicate that the CBC mediates both the activating and the repressing functions of the iron-regulatory transcription factor HapX. The central role of the CBC in environmental adaptation is underlined by HapC-deficiency rendering *A. fumigatus* avirulent in a murine model of aspergillosis. This work was supported by the Austrian Science Foundation grant FWF I282-B09 to HH.

359. Protein kinase A signaling in *Aspergillus fumigatus*: Identification of downstream targets. Juliane Macheleidt[1,4], Wolfgang Schmidt-Heck[2], Ilse D.

Jacobsen[3], Thorsten Heinekamp[1,4], Axel A. Brakhage[1,4]. 1) Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Molecular and Applied Microbiology, Jena, Germany; 2) Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Systems Biology / Bioinformatics, Jena, Germany; 3) Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany; 4) Institute of Microbiology, Friedrich Schiller University, Jena, Germany.

Aspergillus fumigatus is a saprophytic mould normally inhabiting the soil. The fungus also represents a medically important pathogen causing severe systemic infections in immunocompromised patients. To survive in these entirely different habitats, *A. fumigatus* needs mechanisms to sense environmental signals and transduce them intracellularly. One of these signal transduction pathways is the cAMP dependent protein kinase A (PKA) pathway. For *A. fumigatus*, components of this signaling cascade have been characterized in detail and its significance for virulence is evident.

To identify target genes of PKA, we performed microarray analyses using a mutant strain overproducing the PKA catalytic subunit. Following this approach, 23 transcription factors potentially regulated by PKA were identified. From these, 15 were deleted and the mutant phenotypes were characterized. A gene encoding a C6 finger domain protein that showed highest upregulation of all identified transcription factors is located in a potential secondary metabolite gene cluster. Deletion of the transcription factor gene resulted in reduced growth and sporulation of the mutant strain. This phenotype was observed even more drastically for a strain lacking the nonribosomal peptide synthetase of the same cluster. Because genes of this cluster were shown to be transcribed in infected mouse lungs, a virulence study was performed using an embryonated egg infection model. However, the transcription factor deletion mutant showed no altered virulence compared to the corresponding wild type.

To get deeper insights into the function of the secondary metabolite gene cluster, the gene encoding the C6 finger domain protein was overexpressed using an inducible promoter. Overproduction of the transcription factor resulted in induced transcription of all cluster genes and furthermore in the formation of a brown substance which is currently under investigation.

360. *Aspergillus nidulans* galactofuranose biosynthesis affects antifungal drug sensitivity. Md. Kausar Alam, Susan Kaminskyj. Biology, University of Saskatchewan, Saskatoon, SK, Canada.

The cell wall is essential for fungal survival in natural environments. Many fungal wall carbohydrates are absent from humans, so they are a promising source of antifungal drug targets. Galactofuranose (Gal-f) is a sugar that decorates certain carbohydrates and lipids. It comprises about 5% of the *Aspergillus fumigatus* cell wall, and may play a role in systemic aspergillosis. We are studying Aspergillus wall formation in the tractable model system, A. nidulans. Previously we showed single-gene deletions of three sequential *A. nidulans* Gal-f biosynthesis proteins each caused similar hyphal morphogenesis defects and 500-fold reduced colony growth and sporulation. Here, we controlled *A. nidulans ugeA, ugmA* or *ugtA* using the *alcA*(p) or *niiA*(p) promoter. For repression and expression, *alcA*(p)-regulated strains were grown on complete medium with glucose or threonine, whereas *niiA*(p)-regulated strains were grown on minimal medium with ammonium or nitrate. Expression was assessed by qPCR and colony phenotype. The *alcA*(p) and *niiA*(p) strains produced similar effects: colonies resembling wild type for gene expression, and resembling deletion strains for gene repression. Gal-f immunolocalization using the L10 monoclonal antibody showed that *ugmA* deletion and repression phenotypes correlated with loss of hyphal wall Gal-f. None of the gene manipulations affected itraconazole sensitivity, as expected. Deletion of any of *ugmA, ugeA, ugtA*, their repression by alcA(p) or niiA(p), OR, ugmA overexpression by alcA(p), increased sensitivity to Caspofungin. Strains with *alcA*(p)-mediated overexpression of *ugeA* and *ugtA* had lower caspofungin sensitivity. Gal-f appears to play an important role in *A. nidulans* growth and vigor. We are extending these studies to *A. fumigatus* UgmA and UgtA to determine which amino acids are critical for function and Gal-f generation. Previously, we showed that wild type Af*ugmA* can restore an *A. nidulans ugmA* deletion strain to wild type phenotype. Our current results show that certain amino acid residues in *A. fumigatus* UgmA are critical for Gal-f generation: constructs with mutated *A. fumigatus* sequences failed to rescue the An*ugm*D phenotype.

361. WITHDRAWN

362. Sulfur metabolism regulatory mutations induce environmental stress response. J. Brzywczy, M. Sienko, R. Natorff, M. Skoneczny, J. Kruszewska, A. Paszewski. Institute of Biochemistry and Biophysics, 02-106 Warszawa, Poland.

Mutations in the *cysB*, *sconB* and *sconC* genes affect sulfur metabolism in *Aspergillus nidulans* in different ways. The *cysB* mutation blocks synthesis of cysteine and leads to a shortage of this amino acid while *sconB* and *sconC* mutations lead to elevated levels of cysteine and glutathione. We have compared transcriptomes of these three mutants to the wild type strain finding that expression of 1263 genes is altered at least twofold. Transcripts of 908 genes are elevated and 355 genes exhibit decreased levels of transcripts. Despite opposite effect on sulfur metabolism these mutations influence expression of overlapping sets of genes. We have assigned categories of Functional Catalogue to up- and down-regulated genes and have identified categories most enriched with differentially regulated genes. Besides genes involved in sulfur metabolism we find that many up-regulated genes are related to stress responses. Two component signal transduction system is a category that is fifteen times enriched with genes up-regulated in the *sconC* mutant and also highly enriched in the *cysB* and *sconB* mutants (eight- and tenfold, respectively). Genes encoding heat shock proteins and enzymes of glutamate degradation pathway are also up-regulated. The glutamate degradation pathway is also known as a GABA shunt which is induced by an oxidative stress. A large group of up-regulated genes is involved in carbohydrate and energy metabolism including genes coding for enzymes of trehalose and glycerol synthesis. Genes coding for enzymes of alcohol fermentation, which are induced in response to anaerobic stress, are also up-regulated in the sulfur regulatory mutants. Altered expression of carbohydrate metabolism genes is accompanied by changes in sugar accumulation in mutant mycelia and conidia. Among down-regulated genes there are many encoding membrane proteins and enzymes involved in secondary metabolism including penicillin biosynthesis cluster. Genes coding for lysozyme are down-regulated too. As secondary metabolites often inhibit growth of other organisms, lowered expression of genes responsible for their synthesis suggests a decreased response to biotic stress in sulfur metabolism mutants.

363. RNA 3' tagging - signalling transcript degradation and translational repression. Mark X. Caddick, Meriel G Jones, Daniel Rigden, Igor Y Morozov. Dept Biological Sci, Univ Liverpool, Liverpool, Merseyside, United Kingdom.

A large body of work has elucidated the mechanisms associated with mRNA degradation and translational repression - processes which are fundamental to biological systems. For the majority of eukaryotic transcripts, deadenylation is known to lead to transcript degradation and translational repression. However, the surveillance mechanism which determines the point at which functional transcripts are effectively switched off is poorly defined. This transition is generally associated with the mRNA being tagged at the 3' end with a short run of pyrimidine nucleotides. For example, cell cycle-regulated decapping and degradation of mammalian histone mRNA is triggered by uridylation. In fission yeast and the filamentous fungus, Aspergillus nidulans, polyadenylated transcripts are decapped and degraded in response to 3' tagging. In A. nidulans and Arabidopsis tagging involves the addition of a C/U rich element when the transcript's poly(A) tail is shortened to ~15 nucleotides. Disruption of the Aspergillus nucleotidyltransferases, CutA and CutB, results in loss of tagging, lower rates of degradation and an accumulation of transcripts with short poly(A) tails. Recently we have shown that 3' tagging is also

induced by nonsense mediated decay (NMD) and is required for efficient dissociation of the resulting termination complex. Upf1, a major NMD component, is implicated not only in this but, surprisingly, in all mRNA tagging to date. This led us to propose that deadenylation of mRNA results in an "NMD-like" event, where dissociation of poly(A) binding protein results in recruitment of Upf1 to the mRNA thereby triggering translational repression, clearance of the termination complex and efficient RNA degradation. We will describe our recent findings which relate to the mechanisms that trigger mRNA tagging and the downstream events which ultimately repress transcript expression.

364. A novel C2H2 type finger transcription factor, MtfA, regulates mycotoxin biosynthesis and development in *Aspergillus nidulans* . Vellaisamy Ramamoorthy, Sourabh Dhingra, Sourabha Shantappa, Ana M. Calvo-Byrd. Biological Sciences, Northern Illinois University, Dekalb, IL.

Secondary metabolism in the model fungus *Aspergillus nidulans* is controlled by the global conserved regulator VeA, which also governs morphological differentiation. Among the secondary metabolites regulated by VeA is the mycotoxin sterigmatocystin (ST), where the presence of VeA is necessary for the biosynthesis of this carcinogenic compound. We identified revertant mutants (RM) capable of synthesizing ST in the absence of VeA. The point mutation in the RM7 mutant occurred at the coding region of a gene encoding a novel putative C2H2 zinc finger domain type transcription factor that we denominated MtfA. As expected, the *A. nidulans mtfA* gene product localized at nuclei. Deletion of the *mtfA* gene restored mycotoxin biosynthesis in the absence of *veA*, but drastically reduced mycotoxin production when *mtfA* gene expression was altered, by either deletion or overexpression, in the *Aspergillus nidulans* wild type strain. Our study revealed that *mtfA* regulates ST production by controlling the expression of the specific ST gene cluster activator *aflR*. Importantly, *mtfA* also controls sexual and asexual development in *A. nidulans*. Deletion of *mtfA* results in a reduction of conidiation and sexual development.

365. Histidine 704 of the *Aspergillus nidulans* GATA factor AreA is required for nuclear export. Damien Downes[1], Brandon Pfannenstiel[1], Cameron Hunter[1], Kendra Siebert[1], David Clarke[2], Meryl Davis[2], Richard Todd[1]. 1) Department of Plant Pathology, Kansas State University, Manhattan, USA; 2) Department of Genetics, University of Melbourne, Melbourne, AUS.

In *A. nidulans*, the GATA transcriptional activator AreA controls the preferential utilization of nitrogen nutrients as well as the response to nitrogen starvation. During nitrogen starvation AreA accumulates in the nucleus, and a strong increase in target gene expression is observed. Addition of nitrogen nutrients to nitrogen starved cells results in rapid translocation of AreA to the cytoplasm and arrest of elevated AreA-dependent gene expression, indicating that regulated nuclear export is the control mechanism for AreA nuclear accumulation. AreA contains a single conserved CrmA-dependent Nuclear Export Sequence (NES). We propose that regulated AreA nuclear export is controlled by post-translational modification of residues within the NES. We show that deletion of the AreA NES confers nuclear accumulation. Substitution of individual amino acids within the AreA NES identified a single histidine residue, which when mutated to a non-modifiable alanine residue leads to constitutive nuclear accumulation. This suggests that histidine modification may promote AreA nuclear export. We show that fusion of the AreA NES to the constitutively nuclear protein PrnA confers nucleocytoplasmic distribution and a proline utilization loss of function phenotype. We have used this phenotype to select mutants affecting AreA-dependent nuclear export.

366. Redundant Nuclear Localization Signals Mediate Nuclear Import of the Aspergillus nidulans Transcription Activator of Nitrogen Metabolic Genes AreA. Cameron C. Hunter[1], Kendra S. Siebert[1], Damien J. Downes[1], Koon Ho Wong[2], Sara Lewis[2], James A. Fraser[2], David F Clarke[2], Michael J. Hynes[2], Meryl A. Davis[2], Richard B. Todd[1]. 1) Department of Plant Pathology, Kansas State University, Manhattan, KS; 2) Department of Genetics, The University of Melbourne, Parkville VIC 3010, Australia.

The Aspergillus nidulans GATA transcription factor AreA activates transcription of nitrogen metabolic genes. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. AreA contains five putative classical nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS. We used two approaches to identify the functional NLSs. First, we constructed epitope-tagged gene replacement areA mutants affected in individual NLSs or combinations of NLSs to identify sequences required for nuclear localization. Deletion of all five classical NLSs did not affect utilization of nitrogen sources and did not prevent AreA nuclear localization. Mutation of the bipartite NLS conferred inability to utilize alternative nitrogen sources but did not prevent AreA nuclear localization. Combinations of mutations of the six NLSs indicate redundancy among the AreA NLSs. Second, we constructed Green Fluorescent Protein (GFP)-AreA NLS fusion genes and introduced them into A. nidulans. The bipartite NLS strongly directs GFP to the nucleus, one of the classical NLSs weakly directs GFP to the nucleus and the other four classical NLSs collaborate to direct GFP to the nucleus.

367. Conditional expression of the phospho-transmitter gene *ypdA* and the signaling interaction of YpdA with response regulators; SskA and SrrA in *Aspergillus nidulans*. Mayumi Nakayama[1,2], Yura Midorikawa[1,2], Akira Yoshimi[2], Daisuke Hagiwara[2,3], Keietsu Abe[1,2]. 1) Tohoku University, Sendai, Miyagi, Japan; 2) ABE-project New Industry Hatchery Center (NICHe) Tohoku University, Sendai, Japan; 3) Present address: MMRC, Univ. of Chiba, Chiba Japan.

The histidine-to-aspartate (His-Asp) phosphorelay signaling transduction system has been conserved widely in both prokaryotes and eukaryotes. The systems typically consist of three types of common signal transducers: His-kinase (HK), a response regulator (RR), and a histidine-containing phosphotransfer intermediate (HPt). Generally, HPt acts as an intermediate between HK and RR and is indispensable for inducing appropriate responses to environmental stresses through His-Asp phosphotransfer signaling. In *Aspergillus nidulans*, we revealed His-Asp phosphorelay signal transducers: HK (NikA), RR (SskA and SrrA), and HPt (YpdA) were essential for the response of high-osmotic and oxidative stresses. Nevertheless the *ypdA* is the essential gene, the molecular mechanism underlying the importance of YpdA remains unclear. To identify the function of the YpdA, we constructed *A. nidulans* mutant in which expression of the *ypdA* gene is conditionally regulated under the control of the *A. nidulans alcA* promoter (*CypdA* strain) and analyzed their phenotype. We constructed mutant strain from *CypdA* by deleting the response regulator gene *srrA* (*CypdA/DsrrA*) and *sskA* (*CypdA/DsskA*). When *ypdA* was downregulated, *CypdA* showed remarkable growth retardation and formed abnormal hyphae, and *CypdA/DsrrA* unexpectedly showed more severe growth retardation than the parent *CypdA*, in contrast, the growth retardation of *CypdA/DsskA* partly recovered. It is suggested that the growth retardation of *CypdA* was only partly suppressed by switching off the HogA pathway. We further constructed a mutant (*CypdA/DsrrADsskA*) from *CypdA* by deleting the two RR genes. Here, we discuss two-component signaling under the inhibitory conditions of signaling between YpdA and response regulators.

368. WITHDRAWN

369. FigA, a putative member of low-affinity calcium system, is involved in both asexual and sexual differentiation in *Aspergillus nidulans*. Shizhu Zhang, Hailin Zheng, Nanbiao Long, Sha Wang, Ling Lu. College of life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China.

Calcium-mediated signaling pathways are widely employed in eukaryotes and are implicated in the regulation of diverse biological processes. In baker's yeast Saccharomyces cerevisiae, at least two different carrier systems have been identified—a high-affinity calcium influx system (HACS) and a low-affinity

calcium influx system (LACS). In the filamentous fungus Aspergillus nidulans, we identified the homologs of HACS—the voltage gated channel CchA and the stretch activated channel MidA, which formed a complex that played important roles in conidial development, hyphal polarity establishment, and cell wall components in low-calcium environmental condition. In comparison, loss of FigA, a putative member of LACS, showed very severe defects in conidiation and in self-fertility during sexual development under either low or high calcium environmental condition. Interestingly, extracellular Ca2+ was unable to improve these figA defects substantially. Most importantly, the quantitative PCR results revealed the expression of the major asexual development regulator brlA and sexual development regulator nsd, steA had been remarkable regulated in figA mutant. In addition, the localization of Fig1::GFP revealed that FigA was highly accumulated at the center of septum on the mature hypha , and the sites between vesicle-metulae, between metulae-phialide. These data implied that figA likely played important roles in cellular trafficking and communication during in both asexual and sexual differentiation in Aspergillus nidulans. *Correspondence to be addressed: linglu@njnu.edu.cn This work was financially supported by the National Natural Science Foundation of China (NSFC) 31200057 to Z.S, 31070031 to L.L.

370. The *Saccharomyces cerevisiae* FUS3 homologue MAKB in *Aspergillus niger* is a central regulator connecting differentiation and secondary metabolite production with nutrient availability and light. Bert-Ewald Priegnitz, Ulrike Brandt, André Fleissner. Institut für Genetik, TU Braunschweig, Braunschweig, Germany.

Aspergillus niger is an important cell factory in biotechnology. Productivity of this fungus is strongly influenced by the environmental growth conditions and the subsequent morphological adaptation. In eukaryotic organism, mitogen activated protein kinase cascades are important signaling pathways mediating cellular responses to environmental cues. In order to gain a deeper understanding of the molecular mechanisms of cellular adaptation in *A. niger*, we characterized the *Saccharomyces cerevisiae* FUS3 MAP kinase homologue MAKB by using a combination of molecular genetics, biochemical analysis and light and fluorescent microscopy. We found that the absence of makb results in specific developmental defects, which strongly depend on environmental conditions. For example, the mutant secrets a dark pigment in a nutrient dependent manner in constant darkness, but not while growing in a dark-light-rhythm. In addition, MAKB is also involved in asexual spore formation, indicated by its accumulation in developing conidiophores and a reduced sporulation of the makb deletion mutant. Detailed comparison of sporulation in the wild-type and the mutant under different growth conditions let us to the hypothesis that two independent trigger induce conidiation: nutrient starvation and cell age or density. Interestingly, MAKB appears to be only involved in the latter one, indicated by suppressed sporulation of the mutant under nutrient sufficient growth conditions. Taken together, these observations indicate that in *A. niger*, MAKB is intimately involved in the adaptation of secondary metabolism and cell differentiation in response to environmental influences.

371. Engineering and characterizing protein secretion in Aspergillus niger. Y. Zheng, G. Budkewitsch, S. Bourque, J. Burai, N. Geoffrion, J. Richard Albert, E. Munro, S. Sillaots, R. Storms. Biology, Concordia University, Montreal, QC, Canada.

Aspergillus niger is widely used commercially and for basic research as a host for native and foreign protein production. This is mainly because of its ability to secrete large amounts of protein into the growth medium, and carry out the eukaryotic post-translational modifications of glycosylation, proteolytic cleavage and disulfide bond formation. Although A. niger can express some native and foreign proteins at high levels, many native proteins and most foreign proteins are expressed at very low levels. Competition with native proteins for rate limiting steps in the secretory pathway, transcription, mRNA processing and translation are bottlenecks that can limit levels of secreted protein production. Furthermore, proteins expressed using A. niger often require extensive purification, due to the presence of high levels of many native secreted proteins that can interfere with the downstream characterization or reduce protein stability. To address these issues we have been developing recombinant promoters that support high transcription rates, engineering strains that produce reduced levels of native secreted protein, and identifying secretory pathway bottlenecks that limit secreted protein yields. We have created a set of novel expression cassettes that are capable of supporting significantly higher transcription rates than are obtained with the widely used glucoamylase gene (glaA) promoter. We have also, engineered new "clean" expression strains that combine dramatically reduced levels of "contaminating" native extracellular protein production with increased levels of target protein expression. RNA-seq analysis and conditional gene expression are being used to identify additional bottlenecks that limit the production of foreign proteins.

372. Design of culture conditions for secondary metabolite production of fungi based on large-scale transcriptome data. M. Umemura[1], H. Koike[2], M. Sano[3], N. Yamane[2], T. Toda[2], K. Tamano[1], S. Ohashi[3], M. Machida[1]. 1) Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan; 2) Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; 3) Kanazawa Institute of Technology, Kanazawa, Japan.

Fungi produce a wide variety of secondary metabolites, many of which possess bioactivity against bacteria, fungi, and human diseases (ex. penicillin, micafungin, and lovastatin). Inducing the fungal productivity of secondary metabolites is requisite for study and industrial applications of the compounds. Since types and amounts of the secondary metabolites greatly change according to strains and culture conditions, controlling the productivity is difficult and has largely depended on empirical knowledge. More data-based knowledge is required especially when treating the metabolites for which less empirical knowledge has been accumulated. In this study, we have developed an algorithm to analyze metabolic pathway activities using large-scale transcriptome data, and applied it to 72 sets of *Aspergillus oryzae* transcriptome data obtained using different nutritions. Based on the classification of metabolic pathways and assignment of *A. Oryzae* genes to them by Vongsangnak et al. [*BMC Genomics*, 9,245 (2008)], we evaluated the correlations of the pathways and found some positive and negative correlations between primary and secondary ones. We also found that secondary metabolic pathways were divided into two groups depending on the type of nutrition. Combining some culture conditions based on these results, we designed the ideal nutrition for inducing each two groups of secondary metabolic pathways. We are now confirming metabolic pathway activity and secondary metabolite productivity under the designed culture conditions using DNA microarray and LC-MS, respectively.

373. The effect of the *clbR* overexpression on cellulose degrading enzyme production in *Aspergillus aculeatus*. S. Tani, A. Kawamura, E. Kunitake, J. Sumitani, T. Kawaguchi. Life & Environmental Sci, Osaka Prefecture Univ, Osaka, Japan.

Aspergillus aculeatus has two pathways that control the induction of cellulase and hemicellulase genes in response to cellulose. The expression of carboxymethylcellulase 1 (*cmc1*) and FIb-xylanase (*xynIb*) genes was controlled by XlnR, in contrast the expression of cellobiohydrolase I (*cbhI*), carboxymethylcellulase 2 (*cmc2*), hydrocellulase (*cbhIb*) and FIa-xylanase (*xynIa*) genes was controlled by XlnR-independent signaling pathway. We have reported that ClbR, a $Zn(II)_2Cys_6$ transcription factor, controlled the cellulose-responsive induction of the genes regulated by both XlnR-dependent and XlnR-independent signaling pathways. Therefore, we investigated if their enzyme productions could be improved by the *clbR* overexpression. The *clbR* gene was expressed under the promoter of the translation elongation factor 1 alpha gene. Interestingly, xylanase activity in the *clbR* overexpressing strain (clbRox) increased 6-fold more than that in control strain. Although endoglucanase activity did not increase, its activity was kept at high level for 10 days. Peptide mass fingerprinting revealed that FIa-xylanase production was drastically increased in the clbRox strain, in contrast that hydrocellulase production

decreased. Northern blot analysis revealed that the expression of cbhIb decreased in the clbRox strain under cellulose inducing condition. However, the *clbR* overexpression increased xynIa expression under wheat bran inducing condition, but not under the avicel inducing condition. The effect of the *clbR* overexpression was not equal to the enzyme production and the gene expression even though they were under the control of ClbR. These results suggest that ClbR coordinately controls their expression with another factors.

374. Regulation of the a-tubulin B-encoding gene during early development of the phytopathogenic fungus *Botrytis cinerea*. Y. Faivre Talmey[1], I. R. Gonçalves[1], A. Simon[2], M. Viaud[2], C. Bruel[1]. 1) CNRS, University Claude Bernard, LYON, France; 2) INRA, BIOGER, Grignon, France.

The fungal plant pathogen *Botrytis cinerea* is able to infect more than 250 different hosts and is responsible for major economic losses worldwide. The success of the fungus life cycle relies on distinct developmental stages and specific cellular structures like appressoria, infection cushions, conidiophores, apothecia and sclerotia. Based on the important role that cytoskeleton plays in cellular organization and shape in all eucaryotes, attention was given to tubulins and their putative specific role in the virulence of *B. cinerea*. One b-tubulin and two a-tubulin encoding genes are found in B. cinerea's genome. Alpha-tubulin A and B share 69% identity at the protein level and phylogenetic analyses revealed that orthologs to the a-tubulin A gene exist in all ascomyceta species whereas the a-tubulin B gene seems to be present in some ascomyceta only. Expression studies showed that the a-tubulin B gene is more expressed than the a-tubulin A gene. Besides, a peak of expression is observed for the a-tubulin B encoding gene early during conidia-derived fungal development. In order to understand the regulation of the a-tubulin B encoding gene, combinations of promoter deletions and transcriptional fusions were used. Putative regulatory regions were identified and the one hybrid yeast system was used to search for putative transcription factors that would interact with these regions and play a role in the regulation of this gene.

375. The regulation of D-galacturonic acid utilization in Botrytis cinerea. Lisha Zhang, Joost Stassen, Sayantani Chatterjee, Maxim Cornelissen, Jan van Kan. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

The plant cell wall is the first barrier to pathogen invasion. The fungal plant pathogen Botrytis cinerea produces a spectrum of cell wall degrading enzymes for the decomposition of host cell wall polysaccharides and the consumption of the monosaccharides that are released. Especially pectin is an important cell wall component, and the decomposition of pectin by B. cinerea has been extensively studied. D-galacturonic acid is the most abundant component of pectin and effective utilization of D-galacturonic acid is important for virulence of B. cinerea. The D-galacturonic acid catabolic pathway comprises three enzymatic steps, involving D-galacturonate reductase (encoded by Bcgar1 and Bcgar2), L-galactonate dehydratase (encoded by Bclgd1), and 2-keto-3-deoxy-L-galactonate aldolase (encoded by Bclga1). Therefore, an effective concerted action of the appropriate pectin depolymerising enzymes, monosaccharide transporters and catabolic enzymes is important for complete pectin utilization by B. cinerea. In this study, RNA sequencing was performed to compare genome wide transcriptional profiles in B. cinerea grown in media containing glucose and pectate as sole carbon sources. We identified 31 genes that are significantly upregulated in pectate containing culture, including Bcgar2, Bclga1, and a putative monosaccharide transporter. In addition, conserved cis-regulatory elements were predicted in the promoters of genes involved in pectate decomposition and D-galacturonic acid utilization. Functional analysis was carried out of the bidirectional promoter of the Bcgar2-Bclga1 gene cluster to study which of the cis-regulatory elements is required for induction by D-galacturonic acid. Furthermore, potential regulatory protein(s) were isolated by DNA-protein pull down assays using one important cis-regulatory element.

376. Epigenetic Regulation of Subtelomeric Gene Noise in *Candida albicans*. Matthew Z Anderson, Joshua A Baller, Lauren J Wigen, Judith Berman. Genetics, Cell Biology and Development, Univeristy of Minnesota, St Paul, MN.

Candida albicans grows within a wide range of fluctuating host niches, and the ability to rapidly adapt enhances its success as a commensal and as a pathogen. The recently expanded telomere-associated (*TLO*) gene family consists of fourteen expressed members in *C. albicans*. Each *TLO* gene encodes a paralog of a single Mediator complex component. Thirteen expressed *TLOs* are located at the chromosome ends as the most telomere-proximal open reading frame. Individual *TLO* expression at both the transcript and protein level was extremely noisy. Noise originated from single cell variability in *TLO* expression due to intrinsic factors. Deletion of chromatin modifying enzymes that function in subtelomeric silencing abolished *TLO* noise, as did ectopically expressing a *TLO* from an internal locus. Conversely, transcriptional variation of a low noise gene increased significantly when ectopically expressed in the subtelomere. Interestingly, deletion of the Mediator component *MED3*, which inhibits Tlo from incorporating into Mediator, also drastically reduced *TLO* noise and supports an autoregulatory mechanism for *TLO* noise. These data suggest subtelomeric chromatin structure regulates *TLO* gene noise through the action of chromatin modifiers and Mediator. We propose that *TLO* noise is beneficial to *C. albicans* by producing heterogeneous cell populations that incorporate different Tlo proteins in Mediator, producing a range of transcriptional profiles in the population that allows some cells to survive in altered environmental conditions.

377. Signaling and Cell Behavior: Pheromone Response in *Candida albicans*. Ching-Hsuan Lin[1,2], Kabrawala Shail[2], Fox Emily[3], Nobile Clarissa[3], Johnson Alexander[3], Bennett Richard[2]. 1) Dept. Biochem. Sci. and Tech., NTU, Taipei, Taiwan; 2) Dept, MMI, Brown Univ. RI; 3) Dept, Biochem. Biophy. UCSF, CA.

Candida albicans is currently the most common human fungal pathogen, and its propensity for causing infection has been closely lined with its ability to form biofilms. Central to understanding its behavior is the white-opaque phenotypic switch, in which cells can undergo an epigenetic transition between the white state and the opaque state. The phenotypic switch regulates multiple properties including biofilm formation, virulence and sexual mating. In particular, opaque cells are the mating competent form whereas white cells do not mate but generate biofilms in response to pheromone. In this work, we identify the master transcriptional regulator involved in pheromone-induced biofilms in white cells as well as sexual mating in opaque cells as *C. albicans* Cph1, ortholog of *Saccharomyces cerevisiae* Ste12. In contrast, Cph1 is not required for the formation of conventional biofilms (biofilms that can form without pheromone treatment). Transcriptional profiling analysis under biofilm conditions during pheromone treatment revealed a number of potential downstream targets of Cph1 and Tec1. In total, we observed 23 genes that exhibited decreased induction (>2-fold) by pheromone in both *Dcph1* and *Dtec1* mutants. Of these genes, six candidates were chosen for further analysis due to their dependence on Cph1 and Tec1 for pheromone-induced expression, and because they were also not induced in pheromone-treated opaque cells. Interestingly, one novel gene product shown to influence biofilm formation is Hgc1. Similar to the *Dtec1*, deletion of *HGC1* resulted in decreased biofilm formation in white cells responding to pheromone, and also abolished formation of conventional biofilms. It is therefore apparent that Hgc1 is important for cell adhesion and biofilm development in the two distinct models of biofilm formation. Most importantly, these results suggest that both shared and unique components operate in different models of biofilm formation in this important human pathogen.

378. Transcriptional regulatory networks controlling the early hypoxic response in Candida albicans. A. Nantel, M. van het Hoog, A. Sellam, C. Beaurepaire, F. Tebbji, M. Whiteway. National Research Council of Canada, Montreal, Quebec, Canada.

The ability of Candida albicans to colonize or invade multiple host environments requires that it rapidly adapts to different conditions. Our group has been exploiting ChIP-chip and transcription profiling technologies, together with computer modeling, to provide a better understanding of select transcription factor (TF) networks. We used DNA microarrays to measure the changes in transcriptional profiles that occur immediately following the transfer of C. albicans to hypoxic growth conditions. The impressive speed of this response is not compatible with current models of fungal adaptation to hypoxia that depend on the inhibition of sterol and heme biosynthesis. Functional interpretation of these profiles was achieved using Gene Set Enrichment Analysis, a method that determines whether defined groups of genes exhibit a statistically significant bias in their distribution within a ranked gene list. The Sit4p phosphatase, Ccr4p mRNA deacetylase and Sko1p TF were identified as novel regulators of the early hypoxic response. While cells mutated in these regulators exhibit a delay in their transcriptional responses to hypoxia their ability to grow in the absence of oxygen is not impeded. Promoter occupancy data on 26 TFs was combined with the profiles of 375 significantly-modulated target genes in a Network Component Analysis (NCA) to produce a model of the dynamic and highly interconnected TF network that controls this process. The NCA also allowed us to observe correlations between temporal changes in TF activities and the expression of their respective genes, thus allowing us to identify which TFs are potentially subjected to post-transcriptional modifications. The TF network is centered on Tye7p and Upc2p which are associated with many of the genes that exhibit the fastest and strongest up regulations. While Upc2p only associates with downstream promoters, Tye7p is acting as a hub, its own promoter being bound by itself and 7 additional TFs. Rap1 and Ahr1 appear to function as master regulators since they bind to a greater proportion of TF gene promoters, including those of Upc2p and Tye7p. Finally, Cbf1p, Mrr1p and Rap1p show the greatest numbers of unique gene targets. The high connectivity of these models illustrates the challenges that lie in determining the individual contributions of specific TFs.

379. Gene expression and function during invasive Candida infection. Wenjie Xu[1], Norma Solis[2], Carol Woolford[1], Scott Filler[2], Aaron Mitchell[1]. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Departments of Medicine and Pathology, Harbor-UCLA, Los Angeles, CA.

What genes does a pathogen express during infection? What regulatory pathways contribute to expression of those genes in vivo? Which pathogen gene functions evoke specific host responses? These questions are beginning to be addressed for many plant pathogens, in which a lesion that is enriched for infected tissue can be isolated readily. There have also been pioneering studies with human and animal pathogens, but they have been limited by the ability to isolate infected tissue and by background problems from microarray technology. We implemented a recently developed technology, nanoString profiling, to investigate these long-standing questions with the fungal pathogen C. albicans in a murine model of hematogenously disseminated candidiasis. We used whole kidneys, a major target organ, for profiling the time course of both pathogen and host gene expression. NanoString technology is not genome-wide, so we have selected high-priority fungal and host genes for investigation. On the pathogen side, we find that hyphal genes and neutral/alkaline pH response genes are induced early in infection, while oxidative and cell wall stress genes are induced later. These results are consistent with the idea that the influx of neutrophils causes cell wall and oxidative stress. Among the 222 C. albicans genes that specify transcription factors, those highly expressed or highly up-regulated during infection are enriched for previously demonstrated virulence regulators, and also include many genes not previously known to govern virulence. We have profiled attenuated mutants despite their limited growth during infection, and we have found that transcription factor target genes in vivo differ considerably from the target genes identified in vitro. Finally, we find that the host displays progressive induction of cytokines, pattern recognition receptors, and innate immune signaling pathways, with expression changes detectable at 12 hours post-infection, when fungal burden is extremely low. An attenuated rim101 mutant causes a muted host response, but also alters the kinetic profile to yield precocious induction of late host response genes. Our data allow the most detailed sketch to date of the dynamics and functions at the host-pathogen interface during disseminated candidiasis.

380. Effects of histone H3 point mutations on centromere maintenance. Steven Friedman[1], Eric Selker[2], Michael Freitag[1]. 1) Biochemistry & Biophysics, Oregon State University, Corvallis, OR; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR.

Post-translational modifications (PTM) of histone amino acid residues are known to play important roles in chromatin structure and function. In *Neurospora crassa*, trimethylation of histone H3 lysine 9 (H3K9me3) is essential for cytosine DNA methylation [1]. There is also evidence for a role of H3K9me3 in the maintenance of centromeres [2]. An in-depth study of a larger set of histone H3 point mutations revealed additional recessive and dominant mutations involved in DNA methylation, including some mutations that proved lethal [3]. In this study, *hH3* alleles were integrated ectopically or at the *his-3* locus in the presence of a mutant *hH3* allele at the endogenous *hH3* locus. Here we describe a gene replacement system that allows mutant hH3 alleles to be integrated at the endogenous loci by homologous recombination, yielding *hH3* replacement strains. The general approach is versatile and applicable to studying the role of specific point mutations in other genes. We will present results on how *hH3* mutations affect the deposition of centromere proteins (e.g., CenH3, CEN-T and CEN-C).

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381. Analysis of the transcriptional regulation of genes involved in the synthesis and organization of the cell wall of *Ustilago maydis* during infection of an alternatiive host. Angélica Mariana Robledo Briones, José Ruiz Herrera. Centro de Investigaciones y de Estudios Avanzados del IPN, Km 9.6 Libramiento Norte Carretera Irapuato-León.

The cell wall is the most external structure of the cell. Its function is to protect it against the difference in osmotic pressure with the environment and provide the morphology. The wall in fungi is made of microfibrils of structural polysaccharides (chitin and b-1,3-glucans) immersed in a matrix of glycoproteins . *Ustilago maydis* is a dimorphic basidiomycota, pathogen of maize, but under axenic conditions it may infect other plants including Arabidopsis thaliana. Considering the role of the wall in the pathogenic process, we made a transcriptomic analysis of the genes involved in its structure and synthesis, and encoding secreted proteins during the infection of A. thaliana. For this study, one channel chips with high density oligonucleotide were used. Plantlets of Arabidopsis were infected with a haploid or a diploid strain, and at intervals RNA was isolated, complementary cDNA was synthesized and used to hybridize the microarrays. Data of microarrays allowed to identify genes involved in cell wall synthesis and organization, and encoding proteins from the secretome of *U.maydis* whose expression was regulated during the transition from saprophytic to pathogenic stages. These accounted to about 60 per cent; of the total of 639 genes existing in *U. maydis*, a proportion being slightly higher in the haploid strain. Some differences were observed in the regulation of these genes in the haploid and diploid strains. We observed that genes involved in *N*- and *O*- glycosylation of proteins were up-regulated during infection. In addition, some *CHS* and *CDA* genes, and some genes involved in the synthesis of b-1,6-glucans and GPI proteins, were differentially regulated. A great number of genes encoding secreted proteins with a degradative function were up-regulated (more in the haploid). This increased transcription may be related with degradation of the plant cell wall necessary to establish the infection. All these results demonstrate the usefulness of the *Ustilago maydis-Arabidopsis thaliana* pathosysthem for identification of the pathogenic mechanisms of *U. maydis*, and in the case of this

study the role of genes encoding proteins of the wall and secretome, and the differences existing in the behavior of the diploid and haploid, taking into consideration that only the first one is pathogenic to the natural host.

382. Expression of the *Trichophyton rubrum ace2* and *pacC* genes during degradation of keratinized substrates. Larissa Silva, Nalu Peres, Gabriela Persinoti, Elza Lang, Vanderci Oliveira, Antonio Rossi, Nilce Martinez-Rossi. University of Sao Paulo, Ribeirão Preto/SP/Brazil, Sao Paulo, Brazil.

Trichophyton rubrum is a pathogenic, cosmopolitan and anthropophilic fungus that infect keratinized tissues, mainly skin and nails. The genomes of several dermatophytes, including *T. rubrum*, were sequenced by the Broad Institute/NIH, enabling studies on the regulation of the expression of genes related to several cellular processes. The transcription factor (TF) Ace2 participates of a network of genes called RAM (Regulation of Ace2 activity and cellular morphogenesis), involved in the regulation of morphogenesis, cell division, and development of conidiophores. The TF PacC regulates the transcription of genes in response to extracellular pH and also genes related to the biosynthesis of cell wall, suggesting a crosstalk between these two pathways. Therefore, the aim of this study was to analyze the expression profile of the *pacC* and *ace2* genes in different nutritional sources (nail and skin *ex vivo* infections) to understand the regulation of these TF during pathogenesis and development. *In silico* analyses of the putative promoter regions of the *pacC* and *ace2* genes revealed the presence of the DNA binding motifs of both TFs, suggesting a possible cross- and co-regulation of these TFs expression in *T. rubrum*. Gene expression analyses during growth in keratinized tissues suggested an opposite expression profile in nail interaction assays, the *ace2* gene was up-regulated and *pacC* was down-regulated. Moreover, in *ex vivo* skin infective both genes presented a similar expression level. These results suggest a different gene expression modulation of *ace2* and *pacC* according to the nutrient source and possibly the infection site. Moreover, this evaluation provides a better comprehension of the involvement of both pathways in regulating a variety of cellular processes that enable cell viability during infection of keratinized tissues.

383. Control and Function of Two Fatty Acid Regulators in *Neurospora crassa*. Erin L. Bredeweg[1], Fei Yang[2], Kristina Smith[1], Rigzin Dekhang[2], Jillian Emerson[3], Jay Dunlap[3], Deborah Bell-Pedersen[2], Matthew Sachs[2], Michael Freitag[1]. 1) Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing (CGRB), Oregon State University, Corvallis, OR 97331; 2) Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX; 3) Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

The filamentous saprobe *Neurospora crassa* is an excellent model for describing the behavior of transcriptional regulators. We describe the genome-wide behavior of two Fatty Acid Regulators (FAR) proteins, transcription factors that modulate the response of *N. crassa* to the presence of fatty acids. We used ChIP-seq to find the localization of FAR-1 and FAR-2 under nutrient conditions targeting short and long chain fatty acid carbon sources, with sucrose as a control. Bioinformatic analyses describe variant binding sites for FAR-1 and FAR-2, with overlap in about a third of all target regions. Functions under the control of ChIP-seq targets were further examined by phenotypic assays for siderophore production, oxidative stress, and linear growth. We found reduced siderophore production, and increased vulnerability to oxidative stress in *far-1* mutants, but not *far-2* mutants. Linear growth showed a carbon-specific reduced growth rates for *far-2*, as well as Tween-20 sensitivity and conidiation defects for *far-1*. RNA-seq identified numerous differentially regulated transcripts under different growth conditions and in the single or double mutants. Many of these transcripts are part of the gene set identified by ChIP-seq, and many were affected by the absence of one or both FARs. Our analyses identified groups of co-regulated proteins not previously identified as affected by FAR transcription factors, in addition to those involved in the control of the core cellular machinery for energy production by beta-oxidation.

384. Characterization of genomic targets for the *Neurospora crassa* hypothetical transcription factor NCU04390 by ChIP-sequencing. R. Gonçalves[1], E. Bredeweg[2], M. Freitag[2], M. C. Bertolini[1]. 1) Instituto de Química, UNESP, Araraquara, São Paulo, Brazil; 2) Department of Biochemistry and Biophysics, OSU, Corvallis, OR, USA.

The mechanisms by which glycogen content is controlled in microorganisms are intricate, involving co-regulation of many proteins. In *Neurospora crassa*, glycogen reaches maximal levels at the end of the exponential growth phase, however under heat shock, glycogen content and transcription of the glycogen synthase gene (*gsn*) rapidly decrease. In a previous analysis, the NCU04390^{KO} strain showed a drastic increase in glycogen levels and up-regulation of the *gsn* gene after heat shock when compared to the wild-type strain, suggesting that the NCU04390 gene product is involved in the regulation of glycogen metabolism. Because the product of this ORF is annotated as a hypothetical transcription factor (TF) with an N-terminal zinc-finger and a central fungal-specific TF domain, chromatin immunoprecipitation followed by high throughput DNA sequencing (ChIP-seq) was expected to reveal genes that are directly regulated by the NCU04390 gene product. First, GFP tag was fused to the 3'-end of the ORF NCU04390 by gene replacement. ChIP was performed with NCU04390-GFP at 30ºC and 45ºC with antibodies against the GFP tag. ChIP-libraries were sequenced on a HiSeq2000 (Illumina/Solexa) genome analyzer and data from 45ºC experiment revealed that most of the genes regulated by the transcription factor encode hypothetical proteins. However genes encoding proteins with known functions, such as proteins involved in carbon metabolism and transporters were also identified. Among these genes, it is important to mention the glycogen debranching enzyme coding gene (ORF NCU00743), which participates in the glycogen degradation. The His::4390 recombinant protein was produced in *E. coli*, partially purified by IMAC and used in EMSA experiments to validate the result found in the ChIP-Seq assay. The results showed specific binding of the recombinant His::4390 in the NCU00743 promoter, suggesting that the transcription factor might regulate glycogen metabolism under heat stress through the gene encoding the debranching enzyme. Data from EMSA validation analysis for more peaks found in ChIP-seq will be presented. Supported by FAPESP, CNPq, CAPES and US NIH.

385. The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in *Fusarium graminearum*. Kristina M. Smith, Lanelle R. Connolly, Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

The cereal pathogen *Fusarium graminearum* produces secondary metabolites toxic to humans and animals, yet coordinated transcriptional regulation of secondary metabolite gene clusters remains largely a mystery. By ChIP-sequencing we found that regions of the *F. graminearum* genome with secondary metabolite clusters are enriched for a histone modification, trimethylated histone H3 lysine 27 (H3K27me3), associated with gene silencing. This modification was found predominantly in regions that lack synteny with other *Fusarium* species, generally subtelomeric regions. H3K27me3 and di- or trimethylated H3K4 (H3K4me2/3), modifications associated with gene activity, are found in mutually exclusive regions of the genome. To better understand the role of H3K27me3, we deleted the gene for the putative H3K27 methyltransferase, KMT6, a homolog of *Drosophila* Enhancer of zeste, E(z). The *kmt6* mutant lacks H3K27me3, as shown by western blot and ChIP-sequencing, displays growth defects, is sterile, and produces mycotoxins under conditions where they are not generated in wildtype (WT) strains. RNA-sequencing showed that genes modified by H3K27me3 are most often silent, as about 75% of the 4,449 silent genes are enriched for H3K27me3. Surprisingly, we found 22% of the 8,855 expressed genes enriched for H3K27me3. A subset of genes that were enriched for H3K27me3 in WT gained H3K4me2/3 in *kmt6* (1,780 genes), and an overlapping set of genes showed increased

expression. Almost 95% of the remaining 2,720 annotated silent genes showed no enrichment for either H3K27me3 or H3K4me2/3. In these cases absence of H3K27me3 is insufficient for expression, which suggests a requirement for additional factors for gene expression. Taken together, we show that absence of H3K27me3 allows expression of 14% of all annotated genes, resulting in derepression of predominantly secondary metabolite pathways and other species-specific functions, including potentially secreted pathogenicity factors. This study provides the framework for novel targeted strategies to control the "cryptic genome" and specifically secondary metabolite expression.

386. Circadian clock-gated cell division cycles in *Neurospora crassa*. C. Hong[1], J. Zamborszky[1], M. Baek[1], K. Ju[1], H. Lee[1], L. Larrondo[2], A. Goity[2], A. Csikasz-Nagy[3]. 1) Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH; 2) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile; 3) Randall Division of Cell and Molecular Biophysics, and Institute for Mathematical and Molecular Biomedicine, King's College London, London, SE1 UL, UK.

Asynchronous nuclear divisions are readily observed in filamentous fungi such as *Ashbya gossypii* and *Neurospora crassa*. Our computational simulations, however, predict synchronous circadian clock-gated mitotic divisions if the division cycles of such multinucleated organisms are coupled with circadian rhythms. Based on this hypothesis, we investigate the coupling between the cell cycle and the circadian clock in *Neurospora crassa*. First, we show WC-1-dependent light-induced expression of *stk-29* mRNA (homolog of *wee1*), which suggests that there exists a conserved coupling between the clock and the cell cycle via STK-29 in Neurospora as in mammals. Second, we demonstrate that G1 and G2 cyclins, CLN-1 and CLB-1, respectively, show circadian oscillations with luciferase bioluminescence reporters. Moreover, *clb-1* and *stk-29* gene expressions show circadian clock-dependent light-induced phase shifts, which may alter the timing of divisions. Third, we show circadian clock-dependent synchronized nuclear divisions by tracking nuclear morphology with histone *hH1*-GFP reporter. Synchronized divisions occur late in the evening, and they are abolished in the absence of circadian rhythms (frq^{KO}). Our findings demonstrate the importance of circadian rhythms for synchronized mitotic cycles and establish *Neurospora crassa* as an ideal model system to investigate mechanisms that couple the cell cycle and the circadian clock.

387. Protein Binding Microarrays and *high-throughput* real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in *Neurospora crassa*. A. Montenegro-Montero[1], A. Goity[1], C. Olivares-Yañez[1], A. Stevens-Lagos[1], M. Weirauch[2], A. Yang[3], T. Hughes[3], L. F. Larrondo[1]. 1) Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) CAGE, Cincinnati Children's Hospital Medical Center, University of Cincinnati. U.S.A; 3) Banting and Best Department of Medical Research, University of Toronto, Canada.

It has been suggested that ~20% of the Neurospora-transcriptome may be under circadian control. Nevertheless, there is scarce information regarding the regulators that are involved in the rhythmic expression of *clock-controlled genes* (*ccg*s). We are using a *high-throughput* platform, based on various codon-optimized luciferase transcriptional- and translational-reporters, to monitor time-of-day-specific gene expression and to identify key elements mediating circadian transcriptional control. Thus, we have identified transcription factors -such as SUB-1- that affect the expression of known and novel *ccg*s, among which there are transcriptional regulators that give access to a group of third-tier *ccg*s. In addition, we are characterizing several rhythmic bZIP-coding genes as potential nodes of circadian regulation. In order to characterize regulatory networks in which these and all Neurospora transcription factors participate, we are using double-stranded DNA microarrays containing all possible 10-base pair sequences to examine their binding specificities and in that way, predict possible targets on a genome-wide manner. Currently, these Protein Binding Microarray studies have provided DNA-binding specificities for over 120 Neurospora transcription factors granting an unprecedented and powerful tool for transcriptional network studies. Finally, we have generated graphic tools to explore the spatial differences observed in the temporal control of gene expression. Funding: Conicyt/Fondecyt/regular 1090513.

388. Glycogen metabolism is regulated by the circadian clock in *Neurospora crassa*. S. Virgilio, T. Candido, M. C. Bertolini. Instituto de Química, UNESP, Araraquara, São Paulo, Brazil.

The fungus *Neurospora crassa* has been widely used in studies of circadian rhythms and photobiology. Our research group has been using this model organism to study the molecular mechanisms involved in glycogen metabolism regulation and recent findings have revealed that circadian rhythms control a variety of physiological and metabolic functions in different organisms. In a screen of a mutant strains set we identified a number of transcription factors/cofactors likely acting as regulators of glycogen metabolism. Among them, several transcription factors were previously described as controlled by regulators of the circadian clock in *N. crassa*. The result led us to start to investigate whether glycogen metabolism is under control of circadian clock in *N. crassa*. Experiments were performed to verify whether glycogen was rhythmically accumulated in a wild-type strain. In circadian clock experiments, the glycogen content varied according to the circadian rhythm, with cyclical periods ranging from 22 to 24 h. The glycogen synthase activity (GSN) was quantified in the presence and absence of the allosteric activator glucose-6-phosphate (G6P). The -/+ G6P ratio is considered as an index of phosphorylation, higher levels correlating with lower phosphorylation. The GSN phosphorylation was influenced by the biological clock, showing changes in the GSN phosphorylation status along the experiment. The expression of the *gsn* and *gpn* (encoding glycogen phosphorylase) genes was evaluated in the same experiments and in light-induced experiments as well. In circadian clock analysis, the *gsn* and *gpn* transcripts showed rhythmic expression although not as pronounced as the levels of the *ccg-1* transcript (positive control). In light-induced experiments, the levels of glycogen were kept constant during different times after exposure to light, however the expression of the *gsn* and *gpn* genes showed to be delayed light-induced. The results suggested a connection between the energy derived from the glycogen metabolism and the circadian clock in *N. crassa*. Supported by FAPESP and CNPq.

389. Genetic and Molecular Dissection of the Neurospora Circadian Oscillatory System. Qijun Xiang[1], Bin Wang[1], Chandru Mallappa[1], Jennifer Hurley[1], Arko Dasgupta[1], Jennifer Loros[2], Jay Dunlap[1]. 1) Department of Genetics, Dartmouth Medical School, Hanover, NH03755; 2) partment of Biochemistry, Dartmouth Medical School, Hanover, NH03755.

Transcription/ translation feedback loops are central to all eukaryotic circadian clocks. In the circadian oscillator, the negative feedback loop drives periodic expression of proteins that feed back to reduce their own expression. A heterodimer of proteins, WC-1 and WC-2, acts as a transcription factor to drive expression of the frq gene. Its product FRQ dimerizes and forms a complex with another protein FRH. This complex inhibits the activity of the WC heterodimer creating the negative feedback loop. While canonical clock proteins such as FRQ work exclusively in timing, all systems utilize additional proteins performing other functions in the cell. Among these in Neurospora is the essential putative RNA helicase, FRH. A novel, unbiased genetic screen for circadian negative feedback mutants uncovered a point mutation that completely complements the essential functions of FRH yet is totally arrhythmic, thus genetically separating essential functions from clock-associated roles. In other experiments we used mass spectrometry to look for interactors of FRH, FRQ, and to follow posttranslational modifications of these proteins over the day. Although few modifications are found on FRH, FRQ is extensively modified with nearly 100 phosphorylations. By examining the phenotypes of strains bearing mutants that have lost these sites individually and in groups, we begin to see how temporally regulated phosphorylation has opposing effects directly on overt circadian rhythms and FRQ stability. For over 60% of the

confirmed phosphorylation sites, loss of the individual or neighboring sites have no apparent effect on the free running period length, suggesting that sites may work in groups as dynamically regulated charged domains. Some domains promote FRQ stability and lengthen period and other promote turnover and shorten period. Modifications are dynamic such that at near all times of day "FRQ" describes a heterogeneous mix of proteins with the same amino acid sequence but variable and distinguishable structure and surface chemistry. We have also used luciferase as a reporter to follow the FRQ-WC core oscillator under conditions where growth rhythms are manifest. Under these conditions the FRQ/WC oscillator cycles with a normal compensated circadian period length even when the overt rhythm of growth moves out of the circadian range.

390. Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY. Mian Zhou[1], Jinhu Guo[5], Joonseok Cha[1], Michael Chae[1], She Chen[2], Jose Barral[3], Matthew Sachs[4], Yi Liu[1]. 1) Department of Physiology, UT Southwestern Medical Center, Dallas, TX; 2) National Institute of Biological Sciences, Beijing, China; 3) Departments of Neuroscience and Cell Biology and Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX; 4) Departments of Biology, Texas A&M University, College Station, TX; 5) School of Life Sciences, Sun Yat-sen University, Guangzhou, China.

Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation of highly expressed genes 1-3. In addition, codon usage is also implicated in the control of transcription, splicing and RNA structure 4-6. Many genes, however, exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. Alternatively, however, non-optimal codon usage may also have biological significance. The rhythmic expression and the proper function of the Neurospora FREQUENCY (FRQ) protein are essential for circadian clock function. Here, we show that, unlike most genes in Neurospora, frq exhibits non-optimal codon usage across its entire open reading frame. Optimization of frq codon usage results in the abolition of both overt and molecular circadian rhythms. Codon optimization not only increases FRQ expression level but surprisingly, also results in conformational changes in FRQ protein, impaired FRQ phosphorylation, and impaired functions in the circadian feedback loops. These results indicate that non-optimal codon usage of frq is essential for its circadian clock function. Our study provides an example of how non-optimal codon usage is used to regulate protein expression levels and to achieve optimal protein structure and function.

391. Two putative long non-coding RNAs upstream of transcription factor Znf2 may regulate morphogenesis (or dimorphic transition) in *Cryptococcus neoformans*. Nadia Chacko, Linqi Wang, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Cryptococcus neoformans is an opportunistic human pathogen and the causal agent of fungal meningitis, one of the leading causes of death in immunocompromised patients. The virulence of this dimorphic fungus is closely tied to its morphology as the yeast form is pathogenic while the filamentous form is non-pathogenic. The morphological switch from yeast to filament occurs typically during unisexual and bisexual mating but can occur under mating limiting conditions too. Recently we found that transcription factor Znf2 directs morphological transition from yeast-to-filament and its activity is reversely correlated with fungal virulence. The means to increase Znf2 activity, either by activation of its activator or inactivation of its repressors, could be of great value to alleviate cryptococcosis. In a search to identify upstream regulators of *ZNF2*, we screened 60,000 insertional mutants that mimic *znf2*D phenotype. Insertions in two of the selected mutants were found to be in a potential long non-coding RNA located in the intergenic region of *ZNF2*. This lncRNA was named *RZE1*. The *rze1*D mutant phenotype resembles the znf2D mutant phenotype, supporting our hypothesis that *RZE1* functions upstream of *ZNF2*. Interestingly, the expression of RZE1 is increased under host relevant conditions but not under mating-inducing conditions, suggesting that *RZE1* could be involved in the adaptation to the host during infection. Surprisingly the expression of *ZNF2* is only modestly reduced in the *RZE1* insertional mutants, indicating the existence of other potential regulators or non-transcriptional regulation of *ZNF2* by *RZE1*. Further analysis of the intergenic region of *ZNF2* revealed the presence of another lncRNA, which was named *RZE2*. Long ncRNAs are known to regulate genes by transcriptional activation, repression and epigenetic control. The investigation of extent of regulatory role of *RZE1* and *RZE2* on *ZNF2*, the conditions under which they exert regulation, and the mode of action (transcriptional, translational, or epigenetic control) of these ncRNAs will further clarify the role of *ZNF2* in morphogenesis and virulence in *C. neoformans*.

392. Introns in *Cryptococcus neoformans*. Carolin Goebels, Sara Gonzalez-Hilarion, Frédérique Moyrand, Guilhem Janbon. Institut Pasteur, Paris, France.

Cryptoccus neoformans is a encapsulated basiomycetous yeast responsible for deadly meningoencephalitis in immunocompromised patients. The analysis of its genome sequence revealed that nearly all the genes contain introns. These introns are short (67 bp) and each gene contains 5 introns in average. RNAseq data analysis showed that alternative splicing is also very common. Moreover, for most of the genes tested introns appeared to be essential for gene expression. We have studied the pathways by which these introns regulate gene expression in *C. neoformans*. We indentified a polyA binding protein as a major key factor in this regulation controlling the degradation of the mRNA transcribed from intronless alleles.

393. Unravelling of sexual differentiation mediated by Ire1 via Hxl1-independent manners in *Cryptococcus neoformans* . Kwang-Woo Jung, Yong-Sun Bahn. Department of Biotechnology, College of Life science and Biotechnology, Yonsei University, Seoul, South Korea.

Sexual differentiation is a key biological process for generating genetically diverse offspring, which contributes to the increased fitness of certain species in its environmental niches. A human fungal pathogen, *Cryptococcus neoformans*, undergoes both bisexual and unisexual differentiations. Our previous study revealed that UPR (unfolded protein response) components including an evolutionarily conserved ER stress sensor Ire1 and a unique transcription factor Hxl1 modulate ER stress, cell wall integrity, antifungal drug resistance, and virulence in *C. neoformans*. In this study, we for the first time provide several evidences showing that the UPR pathway governs both bisexual and unisexual differentiation of *C. neoformans*. In the serotype A strain backgrounds (H99 and KN99a), the *ire1D* mutants exhibit severe defects in bilateral cross whereas Hxl1 appears to be dispensable for mating in both unilateral and bilateral crosses. Cell fusion efficiency of unilateral and bilateral crossing with *ire1D* mutants is significantly decreased when compared to WT crossing, indicating that Ire1 promotes cell-to-cell fusion during mating. Moreover, deletion of the *IRE1* gene blocked induction of pheromone-mediated conjugation tubes in *crg1D* mutants, which lack a RGS protein that negatively regulates pheromone responsive G-protein signaling. Unexpectedly, however, expression of the mating pheromone gene (*MFa1*) was strongly induced by the mutation of *IRE1* gene in serotype A strain, suggesting that Ire1 has both positive and negative roles in mating of serotype A *C. neoformans*. The *ire1D* mutants constructed in serotype D (JEC21a and JEC20a) strains also exhibit mating defects similar serotype A *ire1D* mutant strains, whereas the *hxl1D* mutants are dispensable for mating. It indicates that the role of Ire1 in sexual differentiation is evolutionary conserved in both serotype A and D strains. The unisexual mating, also known as monokaryotic fruiting, is an alternative differentiation process in *C. neoformans*. The deletion of *IRE1* in the XL280 strain, which is used as a tester strain for same-sex mating, causes significant defects in filamentation whereas the *hxl1D* mutants exhibit levels of filamentation indistinguishable from those of XL280. In conclusion, the Ire1 regulates both bisexual and unisexual mating of *C. neoformans* in Hxl1-independent manners.

394. Functional analysis of PUF mediated post-transcriptional regulation in *Cryptococcus neoformans*. Jan Naseer Kaur, John Panepinto. University at Buffalo, Buffalo, NY.

The Puf (Pumilio and FBF) family of RNA binding proteins is known to bind to 3'UTRs of mRNAs and is associated with regulatory functions including translation, stabilization and localization of transcripts. Investigation of the *C. neoformans* genome has revealed that it encodes four PUF proteins. PUF proteins are typically characterized by the presence of 8 consecutive Puf repeats, however variations do occur. Sequence analysis and evolutionary studies of Puf proteins in fungi has predicted Puf1, Puf2 and Puf3 of *C. neoformans* to contain 5, 3 and 8 PUM-HD repeats respectively. We hypothesize that Puf1, Puf2 and Puf3 act as RBPs and regulate gene expression. The PUF proteins characterized to date have been reported to bind to 3' UTR sequence encompassing a UGUR tetranucleotide in their target RNA. Previous studies have reported that the core motif of UGUA followed by a variable region that Puf3 binds to is conserved from yeast to humans. Scanning of 3'UTRs of all the annotated genes of *C. neoformans* revealed Puf3 binding consensus sequence in the transcripts involved in pheromone signaling cascade. To test this hypothesis we performed bilateral mating assays for wild type and *puf3D* mutants. When equal numbers of opposite mating cells of *puf3D* mutants were cocultured on V8 agar, we observed that they were defective in filamentation as compared to the wild type cross. To determine the ability of *puf3D* mutants to produce pheromone, northern blot was done. The RNA obtained from *puf3D* mutants bilateral cross was probed for mating pheromone MFa in comparison to the wild type cross (H99a x KN99a) and the induction of MFa was found to be normal in *puf3D* mutants mating. Also, fusant colony formation assay revealed that filamention defect of *puf3D* is not due to impaired cell fusion. Using fluorescent microscopy we have shown that mCherry tagged Puf3 localizes to areas of hyphal growth. Our results suggest that mate recognition and fusion do occur when *puf3D* mutants are crossed. We predict that the defect is in Puf3 mediated post fusion hyphal extension. Future studies will determine the mechanism of Puf3 regulation on potential target transcripts. Also we will identify the target/s of Puf1 and Puf2 and their mechanism of regulation which would enable us to establish a link between the physiology and the Puf regulon of *C. neoformans*.

395. Determining the direct targets of two master regulators of sexual development in *Cryptococcus neoformans*. Matthew E Mead[1], Emilia K Kruzel[1], Christina M Hull[1,2]. 1) Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, 53706; 2) Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, 53706.

Cryptococcus neoformans is a major global fungal pathogen that causes disease primarily in immunocompromised individuals. Proposed infectious particles include spores, which are produced as a result of sexual development. In crosses between **a** and a cells this process is in part controlled by the homeodomain transcription factors Sxi1a and Sxi2**a**. To understand the molecular events governing development, we set out to identify direct transcriptional targets of the Sxi1a/Sxi2**a** heterodimer.

First, we created a haploid strain in which galactose-inducible promoters control the expression of *SXI1a* and *SXI2**a***. This *SXI*-inducible strain allowed us to assess global transcript levels in the presence and absence of *SXI* expression. At the same time, we compared changes in transcript levels in crosses between wild type (**a** x a) and *sxi* deletion (*sxi1aD* x *sxi2**a**D*) strains. We discovered that 185 genes exhibited a "Sxi-induced" regulation pattern in both experiments.

Upstream regions of these highly regulated genes were then analyzed using motif-finding algorithms, and a subset of the Sxi-induced genes was found to contain a sequence similar to one bound by Sxi1a/Sxi2**a** in vitro. Individual occurrences of the motif were tested in a Yeast 1-Hybrid system and shown to be bound by Sxi1a/Sxi2**a** in a sequence-specific and heterodimer-specific manner. An in vivo reporter assay was then used to show that these binding sites confer Sxi-dependent regulation to their downstream targets that is also sequence specific.

The list of direct targets studied so far includes numerous uncharacterized genes and putative transcriptional regulators likely important for controlling subsequent developmental transitions. Future studies will focus on building a complete, Sxi-dependent transcriptional network of development. This work will help us better understand a process that results in the production of a likely infectious particle in mammalian disease.

MEM is funded by the Microbes in Health and Disease Training Grant (NIH T32 AI55397).

396. Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in *Cryptococcus neoformans*. Amanda L. Misener Bloom[1,2], Kurtis Downey[1], Nathan K. Wool[1], John C. Panepinto[1,2]. 1) Microbiology/Immunology, SUNY University at Buffalo, Buffalo, NY; 2) Witebsky Center for Microbial Pathogenesis and Immunology, SUNY University at Buffalo, Buffalo, NY.

In response to the hostile host environment, pathogens must undergo rapid reprogramming of gene expression to adapt to the stresses they encounter. Upon exposure to host temperature, Ribosomal protein (RP) transcripts are rapidly repressed in *C. neoformans*. We are interested in investigating specific mechanisms involved in this response, as this repression may be a critical process in host temperature adaptation. Using a mutant null of the major deadenylase, Ccr4, we have discovered that this repression is in part due to enhanced degradation of RP-transcripts. Ccr4 lacks a nucleic acid binding domain and therefore must be recruited to mRNA targets via RNA binding proteins. Using MEME analysis and chromatographic techniques, we have identified a shared cis element in the 3'UTR of RP transcripts that is recognized by the zinc knuckle protein, Gis2. We are currently investigating the importance of this protein-RNA interaction in the expression of RP genes.

Host temperature-induced enhanced degradation of RP transcripts is also dependent on the dissociable RNA polymerase II subunit, Rpb4. Specifically, we demonstrated that in an *rpb4*D mutant, RP-transcript deadenylation is impaired, suggesting that Rpb4 may be required for Ccr4-targeted degradation. In addition, we observed that upon a shift to 37°C, Rpb4 travels from the nucleus to the cytoplasm, supporting a role for Rpb4 in coupling transcription and degradation. Interestingly, this coupling is not restricted to the RP transcripts, as Rpb4 is also involved in enhanced decay of ER stress transcripts following their peak induction, one hour after a shift to host temperature. We have demonstrated that signaling through PKH enhances the degradation of the RP-transcripts in response to host temperature, but not the ER stress transcripts, highlighting the complexity of this system. We report that when transcription and degradation are uncoupled by the loss of Rpb4, growth at host temperature is impaired and virulence in a mouse model of disseminated cryptococcosis is attenuated. Our data suggests that coupling of transcription and degradation via Rpb4 allows the cell to control the intensity and duration of different responses at specific times following exposure to host temperature, contributing to the ability of *C. neoformans* to adapt to this stress.

397. Protein arginine methylation in post-transcriptional gene regulation and stress adaptation of *Cryptococcus neoformans*. J.T. Graham Solomons, Amanda L.M. Bloom, John C. Panepinto. The Department of Microbiology and Immunology, The State University of New York at Buffalo, Buffalo, NY.

Cryptococcus neoformans is environmental fungus that opportunistically infects immune compromised individuals, and is widely studied as a model basidiomycete. The ability to adapt to host temperature is an essential pathogenic trait of *C. neoformans*, and the degradation of mRNA initiated by the major deadenylase Ccr4 appears to play an integral role in the temperature stress response of *C. neoformans*. Microarray analysis revealed that the largest functionally related group of mRNA stabilized in the ccr4D mutant encode ribosomal protein (RP) transcripts. An RNA-binding protein, identified as Gis2, has been shown to interact specifically with a *cis* element in the 3'UTR of RP transcripts. Gis2 is a small protein (19kDa), predominantly comprised of a

series of a zinc knuckles and a single glycine-arginine rich (GAR) region, which may serve as a target for protein arginine methyltransferases (RMTs). Primary sequence analysis revealed that, Gis2 from *C. neoformans* (CnGis2) shares significant primary sequence identity with Gis2 from *S. cerevisiae* (ScGis2) and the human CNBP/ZNF9 protein. However, ScGis2 does not contain a GAR motif; in contrast, the human ortholog contains a GAR element, and has been shown to undergo methylation by RMT5. An in vitro methylation assay demonstrated that recombinant CnGis2 can be methylated by one (or more) Cryptococcal RMTs. Investigation of the *C. neoformans* genome revealed there are 5 putative RMT genes. A *C. neoformans rmt5*D deletion mutant exhibited a severe growth defect and aberrant cell morphology at 39°C, suggesting that Rmt5 activity impacts temperature adaptation in *C. neoformans*. Further analysis of the rmt5D mutant and the remaining 4 *C. neoformans* RMTs will determine to contribution of protein arginine methylation to the virulence and stress tolerance of *C. neoformans*.

398. UVE1 is a Photo-regulated Gene Required for the Protection of Mitochondrial DNA in Cryptococcus neoformans from UV Induced DNA Damage. Surbhi Verma, Alexander Idnurm. School Of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110.

The UVE1 gene is an apurinic/apyrimidinic endonuclease, identified in a T-DNA insertion mutagenesis screen in the pathogenic fungus Cryptococcus neoformans. UVE1 mutation or deletion leads to a UV hypersensitive phenotype. The homologous gene in fission yeast Schizosaccharomyces pombe encodes apurinic/apyrimidinic endonuclease acting in the UVDE-dependent excision repair (UVER) pathway. C. neoformans UVE1 complements a S. pombe uvde knockout strain, hence functionally similar. In Cryptococcus, the Bwc1-Bwc2 photoreceptor complex regulates mating, virulence and ultraviolet radiation (UV) stress tolerance. How the Bwc1-Bwc2 complex regulates these functions is not clear. We discovered that UVE1 is photoregulated in Bwc1-Bwc2 dependent manner in Cryptococcus, and in Neurospora crassa and Phycomyces blakesleeanus that represent two other major lineages in the fungi. Overexpression of UVE1 in bwc1 mutants rescues their UV sensitivity phenotype and gel mobility shift experiments show binding of Bwc2 to the UVE1 promoter. These experiments indicate that UVE1 is a direct downstream target for the Bwc1-Bwc2 complex, required for UV stress tolerance. Uve1-GFP fusions localize to the mitochondria in C. neoformans. Hence in Cryptococcus, UVE1 is a key photo-regulated gene responsible for tolerance to UV stress for protection of the mitochondrial genome.

399. Uve1 endonuclease protects *Cryptococcus neoformans* from UV damage through regulation by the White collar complex. Surbhi Verma, Alexander Idnurm. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO.

In *Cryptococcus neoformans* the Bwc1-Bwc2 photoreceptor complex regulates mating, virulence and ultraviolet radiation (UV) stress tolerance. We identify and characterize a gene, *UVE1*, whose mutation leads to a UV hypersensitive phenotype. The homologous gene in *Schizosaccharomyces pombe* encodes a apurinic/apyrimidinic endonuclease acting in the UVDE-dependent excision repair (UVER) pathway. *C. neoformans UVE1* complements a *S. pombe uvde* knockout strain. *UVE1* is photoregulated, in a Bwc1-dependent manner in *Cryptococcus*, as well as in *Neurospora crassa* and *Phycomyces blakesleeanus*. Overexpression of *UVE1* in *bwc1* mutants rescues their UV sensitivity phenotype and gel mobility shift experiments show binding of Bwc2 to the *UVE1* promoter, indicating that *UVE1* is a direct downstream target for the Bwc1-Bwc2 complex. Uve1-GFP fusions localize to the mitochondria. In *C. neoformans UVE1* is a key gene regulated in response to light that is responsible for tolerance to UV stress for protection of the mitochondrial genome.

400. Multiple laccase genes in *Schizophyllum commune*. S. Madhavan, K. Krause, E. Kothe. Microbiology Microbial Communication, Friedrich Schiller University, Jena, Germany 07743.

The saprophytic white rot fungus *S. commune*, is involved in the degradation of complex organic molecules including lignin as well as refractory organic matter from black slate with the help of different exoenzymes. Thus, the genome sequence of *S. commune* was used to gain an insight into the functional analysis of laccases and laccase-like enzymes. Laccases are multi-copper oxidases that catalyze oxidation of a wide spectrum of organic and inorganic substances. In most fungi, laccases are found to be multigene families producing isoenzymes with multiple functions. Two laccases and four laccase-like genes have been identified from the genome of *S. commune*. Differential regulation of individual genes was analysed at the transcript level by quantitative real-time PCR. Individual laccase genes showed distinct expression profiles during fungal development, morphogenesis and during substrate utilization. Genes *lcc1* and *lcc4* seem to play a role during the primordial formation for fruiting bodies phase and *lcc2* and *lcc6* were found to be related to the dikaryotic phase and vegetative growth. Gene *lcc5* is regulated during fruitbody formation. Various stress responsive elements (XRE, STRE) could be detected in all respective promoters indicating an infuence of aromatic compounds and stress molecules in transcriptional regulation. Characterization of laccase mutants with respect to organic matter and black slate degradation is linking these data to different functions of single laccase and laccase-like genes.

401. Regulation of DNA repair genes expression by UV stress in *Neurospora crassa*. Tsukasa Takahashi, Makoto Fujimura, Akihiko Ichiishi. Faculty of Life Science, Toyo University, Ora-gun, Gunma, Japan.

In all organisms, DNA is constantly damaged by endogenous and exogenous factors such as environments and chemicals. In these genotoxins, ultraviolet (UV) irradiation induces DNA damage such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). *N.crassa* has three mechanisms to repair UV-damaged DNA; nucleotide excision repair (NER), UV dependent repair (UVDR), photoreactivation (PR). Because UV-induced DNA lesions are efficiently removed by these DNA repair systems, *N.crassa* shows highly resistance to UV as compared to other species. In human and *S.cerevisiae*, it has been reported that some of DNA repair genes involved in removal of UV-damaged DNA were induced by UV irradiation. Furthermore, some MAP kinase pathways were activated in response to UV irradiation in human. In *N.crassa*, characterizations of DNA repair gene mutants have been performed in detail, but relationship between expression of these genes and UV stress are not clear yet. Thus, we investigated whether UV stress is involved in regulation of expression of DNA repair genes, and whether UV stress activates MAP kinase pathway like a human. We show that some DNA repair genes such as *mus-40*, *mus-43* were up-regulated by UV irradiation. The OS-2 MAP kinase, involved in response to osmotic stress in *N.crassa*, was activated by UV irradiation, and then expression of *mus-40*, *mus-43* were not induced after UV irradiation in *os-2* mutant. In addition, *os-2* mutant was more sensitive to UV irradiation than the wild-type. These data suggest that UV stress upregulates some DNA repair genes and UV signal was partially transmitted by OS MAP kinase cascade, in *N.crassa*.

402. Diverse classes of small RNAs originating from genomic hotspots, tRNA and the mitochondrial genome in Phytophthora infestans. Sultana N. Jahan[1], Anna K. M. Åsman[1], Ramesh R. Vetukuri[1], Anna O. Avrova[2], Stephen C. Whisson[2], Christina Dixelius[1]. 1) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO-Box 7080, SE-75007, Uppsala, Sweden; 2) Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, United Kingdom.

Phytophthora infestans is the oomycete pathogen responsible for the devastating late blight disease on potato and tomato. P. infestans is notorious for its ability to evolve to overcome resistant potato varieties. The genome of this pathogen has been sequenced and revealed vast numbers of transposon sequences, and hundreds of disease-promoting effector proteins. We are aiming at understanding gene-silencing mechanisms in P. infestans including deciphering roles of small non-coding RNAs. In our previous work we have shown that P. infestans has an active RNA silencing pathway (Vetukuri et al. 2011). We also performed deep sequencing of sRNAs from P. infestans and knocked down the genes encoding the RNA silencing components Argonaute and Dicer in order to investigate their roles in sRNA biogenesis (Vetukuri et al. 2012). Here, we describe the distribution of genomic sites from which sRNAs originate. Genome-wide analysis of sRNAs revealed diverse classes mapping to genomic sources such as tRNAs, rRNAs, genomic sRNA 'hotspots', and the mitochondrial genome. Most tRNA-derived RNA fragments (tRFs) mapped to the sense strand of the 5'-halves of mature tRNAs and peaked at 27 and 30 nt lengths. In accordance with reports from other organisms (Franzén et al. 2011), the tRFs mapped to different tRNA isoacceptors with unequal frequencies, the Ile_tRNA_Cluster_0 showing the highest proportion of mapping sRNAs. We are presently using our Dicer knockdown transformants to investigate the tRF biogenesis mechanism. Another interesting group of sRNAs are those that map to transposons that have close-by neighboring RXLR-effector genes.

One such example is PiAvr2, which is located just 231 bp from a class II transposon. The presence of sRNAs mapping to both PiAvr2 and the nearby transposon indicate that RNA silencing may play a role in regulation of this important effector gene. Over a hundred additional predicted genes were found to be sRNA hotspots in our data: Crinkler effector genes, arrays of duplicated genes, potentially antisense overlapping transcripts, and genes containing transposon insertions. Our present task is to reveal the role that sRNAs might play in their regulation.

403. Epigenetic control of effector gene expression in the plant pathogen fungus *Leptosphaeria maculans*. Jessica Soyer, Mennat El Ghalid, Marie-Hélène Balesdent, Thierry Rouxel, Isabelle Fudal. INRA, UR 1290 BIOGER-CPP, Avenue Lucien Brétignière, F-78850 Thiverval-Grignon, France.

Plant pathogenic microbes secrete an arsenal of small secreted proteins (SSPs) acting as effectors that modulate host immunity to facilitate infection. In Eukaryotic phytopathogens, SSP-encoding genes are often located in particular genomic environments and show waves of concerted expression at diverse stages of plant infection. To date, little is known about the regulation of their expression. *Leptosphaeria maculans* is an ascomycete fungus responsible for the most devastating disease of oilseed rape (*Brassica napus*). The sequencing of its genome revealed a bipartite structure alternating gene rich GC-isochores and gene poor AT-isochores made up of mosaics of transposable elements. The AT-isochores encompass one third of the genome and are enriched in putative effector genes that present the same expression pattern (no or a low expression level during *in vitro* growth and a strong over-expression during primary infection). Here, we investigated the involvement of an epigenetic control in the regulation of effector gene expression. For this purpose, we silenced expression of two key players of heterochromatin remodeling, *i.e.* HP1 and DIM5, by RNAi and used HP1::GFP as a heterochromatin marker. Whole genome oligoarrays were done in silenced-*HP1* and silenced-*DIM5* isolates to analyze the involvement of HP1 and DIM5 on gene expression according to their function and location. We evaluated the effect of a change of genomic context from AT-isochores to GC-isochores on the expression of effector genes. Silencing of *DIM5* resulted in lack of chromatin condensation. The silencing of *HP1* and *DIM5* resulted in an over-expression of pathogenicity-related genes during *in vitro* growth, with a favored influence on SSP-encoding genes in AT-isochores. The "moving" of effector genes corroborated transcriptomic analysis as it led to a strong overexpression of effector genes during *in vitro* growth. These data strongly suggest that an epigenetic control represses the expression of effector genes located in AT-isochores during in vitro growth, which is, to our knowledge, the first description of an epigenetic control, relying on HP1 and DIM5, exerted on effector-encoding genes expression. Switch toward pathogenesis lifts this repression based on chromatin-structure, rendering promoters of effector genes accessible to specific transcription factors.

404. Discovering the link: The NOX-GSA network for sexual development and ascospore germination in *Sordaria macrospora*. Daniela Dirschnabel, Christian Schäfers, Ines Teichert, Ulrich Kück. General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany.

Recently we were successful in establishing a genetic network for sexual development and ascospore germination in the homothallic filamentous fungus *Sordaria macrospora* [1, 2]. Central components of this network are three G-protein *alpha* subunits (GSA), an adenylat cyclase SAC1, and the transcription factor STE12. The three GSA proteins (GSA1, GSA2 and GSA3) have different roles in developmental processes. GSA1 and GSA2 are important for sexual propagation and the generation of perithecia, while GSA3 is essential for proper ascospore germination. Interestingly, the phenotypes of mutants lacking fungal NAD(P)H oxidases (NOX) resemble the known Dgsa phenotypes: DnoxA shows an arrest of sexual development and ascospores from a DnoxB mutant fail to germinate. These similarities raised the question, whether the GSA proteins and NOX enzymes are part of identical signaling pathways. To verify this hypothesis, we generated knockout mutants of both NOX A and B isoforms and their regulator NOXR in *S. macrospora*. Our hypothesis was further supported by the comparison of these mutants with gsa deletion mutants by measuring hyphal fusion events, quantification of reactive oxygen species and ascospore germination. The generation of double mutants and complementation studies with constitutive *gsa1* derivatives enabled us to propose an interactive NOX-GSA network for sexual development and ascospore germination. References: 1.Kamerewerd, J., M. Jansson, M. Nowrousian, S. Pöggeler, and U. Kück, Three *alpha*-subunits of heterotrimeric G proteins and an adenylyl cyclase have distinct roles in fruiting body development in the homothallic fungus *Sordaria macrospora*. Genetics, 2008. 180(1): p. 191-206. 2.Engh, I., M. Nowrousian, and U. Kück, *Sordaria macrospora*, a model organism to study fungal cellular development. European journal of cell biology, 2010. 89(12): p. 864-72.

405. The bZIP transcription factor Atf1 acts as a global regulator for secondary metabolite production in *Fusarium fujikuro*i. Sabine E. Albermann, Bettina Tudzynski. IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany.

The activating transcription factor 1 (Atf1) belongs to the bZIP transcription factor family and is known to have a great impact on stress responses mediated by the mitogen activated protein kinase (MAPK) cascade in fission yeast. In this pathway, activation of the transcription factor is achieved by phosphorylation via the kinase Sty1. Furthermore, the transcription factor plays a role in sexual and asexual development which was observed for several filamentous fungi *e.g.* in *Aspergillus* species where it affects conidiospore germination. Atf1 can also act as a virulence factor which was described for its homologue in the rye pathogen *Claviceps purpurea*. However, involvement of Atf1 in secondary metabolism was first observed in the grey mould *Botrytis cinerea*. As Atf1 seems to play a crucial role in different processes, this transcription factor was also investigated in the rice pathogen *Fusarium fujikuroi*. For this purpose, deletion mutants of atf1 and the Sty1 homologue *sak1*, the putative kinase for Atf1, were cultivated under varying conditions. HPLC analysis of the secondary metabolite spectrum revealed a drastic change in the production level of several metabolites. Gibberellic acids, for instance, are down-regulated up to 50 % in D*atf1* compared to the wild-type, whereas the amount of gibberellins in the D*sak* mutant is about twice as much as in the wild-type. Furthermore, applied salt stress dramatically enhances mycotoxin production in the D*atf1* mutants, while the deletion mutant D*sak1* is not able to grow at all. Plate assays applying different stressors to the strains revealed involvement of both proteins in the osmotic stress response. However, reactive oxygen species and cell wall damaging agents do not seem to have an impact on their growth. In contrast, reduced protoplast formation was observed for D*atf1* mutants and even more significantly in D*sak*. Therefore, it is very likely, that the cell wall composition and integrity is changed in these mutants. Summarizing, Atf1 and Sak1 are involved in various processes such as secondary metabolite production, cell wall integrity as well as in stress responses. The obtained information leads to the conclusion that Sak1 might be the kinase responsible for Atf1 phosphorylation. But there certainly have to be more factors to be involved in activation of this transcription factor.

406. Role of the Vivid ortholog of Fusarium fujikuroi VvdA in carotenoid biosynthesis and development. Marta Castrillo Jimenez, J. Avalos. Genetics, University of Sevilla, Sevilla, Seville, Spain.

Fusarium fujikuroi is well known for its ability to produce gibberellins, growth-promoting plant hormones with agricultural applications. Recently, this specie has become a model system in the research of other metabolic pathways, including carotenoid biosynthesis. This fungus produces an acidic apocarotenoid, neurosporaxanthin, through the activity of the enzymes encoded by five structural genes, whose expression is induced by light. We are interested in the molecular basis of this regulation. As usually found in fungi, the *F. fujikuroi* genome contains genes for WC-1 and WC-2 orthologs. In contrast to other species with light-induced carotenogenesis, e.g., *Neurospora crassa* or *Phycomyces blakesleeanus*, this photoresponse is not impaired in null mutants of the only *wc-1*-like gene of *F. fujikuroi*, *wcoA*. Therefore, we are analyzing the role of other blue-light photoreceptors. Here we described

the identification, regulation and targeted mutation of the gene *vvdA*, ortholog of the *N. crassa vivid* (*vvd*) gene. The predicted *F. fujikuroi* VvdA protein is similar to VVD in size (198 aa compared to 186 aa) and sequence (87 identical positions). Deletion of *vvdA* in *F. fujikuroi* results in a significant reduction in pigmentation and carotenoid production, a regulatory effect opposite to the enhanced carotenoid accumulation characteristic of the vvd mutants of *N. crassa*. Additionally, *vvdA* mutant colonies exhibit a different aspect in the light, apparently due to more compact development or aerial mycelia. As found for *vvd* in *N. crassa*, expression of *vvdA* in *F. fujikuroi* cultures is strongly stimulated by light, an activation which is severely reduced in the *wcoA* mutants. Accordingly, the alterations exhibited by the *vvdA* mutants are only apparent under illumination. Our results suggest that VvdA participates in the photoreceptor machinery responsible for carotenoid photoinduction in *F. fujikuroi*.

407. Gene expression of secondary metabolism gene clusters by different Fusarium species during in planta infection. J. Espino[1], M. Muensterkoetter[2], U. Gueldener[2], B. Tudzynski[1]. 1) Institut of Plant Biology and Biotechnology, Westf. Wilhelms University,Schlossplatz 8, 48143 Muenster, Germany; 2) 2Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Muenchen (GmbH), Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany.

The Gibberella fujikuroi complex (GFC) comprises about 50 Fusarium species with similar characteristics, which are responsible for an array of plant diseases, causing devastating losses in agriculture. The majority of their members is able to produce different toxins which can contaminate food and feed worldwide. Despite of their similarities, they differ in their spectrum and amount of secondary metabolites (SM) production, probably due to different natural hosts. For example, Fusarium verticillioides is considered as a fumonisin producer and attacks mainly maize, whereas Fusarium fujikuroi causes the bakanae disease in rice, secreting the phytohormone gibberellin beside several other products. Another fungus, Fusarium mangiferae, causes the mango malformation and is neither able to produce fumonisins nor gibberellins. And Fusarium proliferatum produces a very broad spectrum of mycotoxins and infects mainly maize. In the present study we compared the in planta expression profiles for different secondary metabolism gene clusters in these four species of the GFC, and also the one of Fusarium oxysporum as an outgroup not belonging to the GFC. So far, gene expression studies have been done for these fungi mainly in vitro, showing differential regulation mechanisms, e.g. in response to nitrogen availability. But not much is known about the gene expression during plant infection. We have performed an infection assay in maize and rice and quantified the fungal biomass in the roots by quantitative PCR using genomic DNA to determine the ratio between plant and fungal biomass in infected tissue. The expression of SM genes was followed up in time course experiments. The results of this study show differences between the species regarding colonization of the host and expression of SM. Surprisingly the high expression of some gene clusters, which were never expressed before in vitro, suggests a specific induction by plant signals.

408. A *cis*-acting factor modulating the transcription of *FUM1* in *Fusarium verticillioides*. Valeria Montis[1], Matias Pasquali[2], Ivan Visentin[1], Petr Karlovsky[3], Francesca Cardinale[1]. 1) Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, 10095 Grugliasco (TO), Italy; 2) Environment and Agrobiotechnology Dept, CRP GABRIEL LIPPMANN, Belvaux, Luxembourg; 3) Department of Crop Sciences, Molecular Phytopathology and Mycotoxin Research, University of Göttingen, D-37077 Göttingen, Germany.

Fumonisins-biosynthetic FUM genes are clustered and co-expressed in toxin producers. By overrepresentation analyses, we identified a motif in promoters of clustered FUM genes in both fumonisins producers *F. verticillioides* and *A. niger*. The same motif was not found in various FUM gene homologues of fungi that do not produce fumonisins. Deletion of the main 6-mer in FvFUM1 promoter compromises its gene expression both *in planta* and *in vitro*. We hypothesize that such motif may be important for clustered FUM genes coordinated transcription, being the core of a transcription factor-binding site for a putative Zn-finger protein.

409. Shedding light on secondary metabolite cluster gene expression, sporulation, UV-damage repair and carotenogenesis in the rice pathogen *Fusarium fujikuroi*. Phillipp Wiemann, Bettina Tudzynski. Institut für Biologie und Biotechnologie der Pflanzen Westfälische Wilhelms-Universität Münster Schlossplatz 8 48143 Münster Germany.

The rice pathogen *Fusarium fujikuroi* produces economically important secondary metabolites like gibberellic acids and carotenoids as well as mycotoxins like bikaverin and fusarin C. Their production is activated in response to environmental stimuli such as light, pH or nutrient availability. In this study, we evaluate the effects of light and different putative light receptors on growth and differentiation as well as secondary metabolism. Bimolecular fluorescence complementation proved that homologs of the *Neurospora crassa* White Collar proteins in *F. fujikuroi* (WcoA and WcoB) form a nuclear localized complex (WCC) that is needed for full functionality. Deletion and complementation of both genes revealed that the WCC represses bikaverin gene expression in constant light conditions and induces immediate light-dependent carotenoid gene expression as shown by northern blot analyses. Additionally the WCC represses conidiogenesis in response to light. The effects observed regarding bikaverin and carotenoid gene expressions as well as conidiogenesis are antagonistically to the ones observed in the velvet mutant, making a connection between the WCC and the *velvet* complex feasible, similarly to the situation in *Aspergillus nidulans*. Since carotenoid production was maintained in both *wcoA* and *wcoB* single as well as in *wcoA/B* double mutants in constant light conditions, we focused on characterization of additional putative light receptors in *F. fujikuroi*. Deletion of the phytochrome-like-encoding gene *fph1* did not show any significant phenotype. Deletion of *phl1*, coding for a cryptochrome/photolyase demonstrated impaired carotenoid biosynthesis gene expression upon exposure to light. Additionally, gene expression and HPLC analyses of these mutants demonstrated loss of fusarin C gene expression and concomitant production formation compared to the wild type, suggesting a distinct transcriptional activity for this barely characterized class of enzymes. Finally UV mutagenesis experiments and qRT-PCR demonstrate that WcoA, WcoB and Phl1 are involved in UV-damage repair most likely by transcriptionally activating *phr1*, encoding a CPD-photolyase. The data presented here allow us to draw a first model of how light receptors function in a signaling network in the rice pathogen *F. fujikuroi*.

410. Fgap1-mediated response to oxidative stress in trichothecene-producing *Fusarium graminearum*. M. Montibus, N. Ponts, E. Zehraoui, F. Richard-Forget, C. Barreau. INRA, UR1264-MycSA, BP81, F-33883 Villenave d'Ornon, France.

The filamentous fungus *Fusarium graminearum* infects cereals and corn. It is one of the main causal agent of "*Fusarium* Head Blight" and "Maize Ear Rot". During infection, it produces mycotoxins belonging to the trichothecenes family that accumulate in the grains. Although the biosynthetic pathway involving specific *Tri* genes has been elucidated, the global regulation of toxin biosynthesis remains enigmatic. It is now established that oxidative stress modulates the production of toxins by *F. graminearum*. H_2O_2 added in liquid cultures of this fungus enhances trichothecenes accumulation and increases *Tri* genes expression. Our working hypothesis is that a transcription factor regulates redox homeostasis, and is involved in *Tri* genes regulation. In the yeast *Saccharomyces cerevisiae*, the transcription factor Yap1p mediates response to oxidative stress *via* nuclear re-localization and activation of genes coding for detoxification enzymes. In this study, we investigate the role of Yap1p homolog in *F. graminearum*, Fgap1, in response to oxidative stress and its eventual role in the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in *F. graminearum* were constructed. We cultured these mutants in GYEP liquid medium supplemented with H_2O_2 to evaluate their sensitivity to oxidative stress and analyse their toxin production. The nuclear localization of constitutively activated Fgap1p as well as wild-type Fgap1p under oxidative stress by

H_2O_2 was analyzed. Expression profiles of genes encoding oxidative stress response enzymes potentially controlled by Fgap1p and of genes involved in the biosynthesis of type B trichothecenes were analyzed by Q-RT-PCR. Trichothecene accumulation is strongly enhanced in the deleted strain, with an increase in *Tri* genes expression. On the other hand, *Tri* genes expression and toxin accumulation are drastically repressed in the mutant in which Fgap1p is constitutively activated. Moreover, the level of expression of two genes encoding catalases is modulated in both mutants. The involvement of Fgap1 in other types of stress has also been investigated. In particular, cadmium and osmotic stress affect growth in the deleted strain.

411. Functional analyses of *FgLaeA* in *Fusarium graminearum*. Hee-Kyoung Kim, Seong-mi Jo, Seunghoon Lee, Sung-Hwan Yun. Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam 336-745, South Korea.

Fusarium graminearum (telomorph: *Gibberella zeae*) is the casual agent of the head blight of cereal crops and produces mycotoxins such as trichothecenes and zearalenone in infected plants. The expression of genes involved in biosyntheses of these mycotoxins are controlled at the different levels ranging from by a pathway-specific transcription regulator (encoded by *TRI6* or *ZEB2*) to by a global regulator involved in chromatin remodeling. Here we focused on the function of *FgLaeA* in *F. graminearum*, which is an ortholog of the *Aspergillus nidulans LaeA*, encoding the global regulator for both secondary metabolism and sexual development. For functional analysis of *FgLaeA* in mycotoxin production, we used a transgenic *F. graminearum* strain expressing a firefly luciferase gene under control of *TRI6* or *ZEB2* promoter as a reporter system. Targeted deletion of *FgLaeA* led to a dramatic reduction of luminescence in the reporter strain, indicating that *FgLaeA* controls the expression of both *TRI6* and *ZEB2* in *F. graminearum*; the reduced toxin accumulation was further confirmed by HPLC analysis. In addition, the *FgLaeA* deletion strains exhibited not only albino phenotype on CM medium but also earlier formation of sexual fruiting bodies (perithecia) on carrot agar than its wild-type progenitor, the latter indicating that *FgLaeA* seems to negatively control the perithecial induction. Quantitative real-time PCR revealed that *FgLaeA* was expressed constitutively under both mycotoxin production and sexual development. Overexpression of a *GFP-FgLaeA* fusion construct in a *FgLaeA*-deletion strain recovered all the phenotypic changes to the wild-type levels, and led to constitutive expression of GFP in the entire cells at different developmental stages. A split luciferase assay for in vivo protein-protein interaction demonstrated that FgLaeA could not interact with FgveA, an ortholog of *A. nidulans* veA. Taken together, it is likely that *FgLaeA* controls both secondary metabolism and sexual development in *F. graminearum*, but the regulation pattern operated by *FgLaeA* is somewhat different from that by *LaeA* in *A. nidulans*.

412. Molecular cloning and differential expression of two novel Family 1 b-glucosidases genes from the rare fungus Stachybotrys microspora. Salma Abdeljalil, Houcine Lazzez, Ali Gargouri. Centre of Biotechnology of Sfax, Sfax-Tunisia.

The cellulolytic system of the fungus Stacchybotrys microspora is characterized by the existence of several b-glucosidases. From a compilation of fungal b-glucosidases belonging to family GH1, we designed primers to isolate b-glucosidases by PCR. Using different primers combination, three different fragments genes were firstly obtained. Two of them are overlapping and constitute a novel gene named Smbgl1A while the third one is a part of a second gene named Smbgl1B. RT-PCR analysis showed the first gene is induced by cellulose and repressed by glucose while Smbgl1B is equally expressed on both conditions The identification of putative catalytic residues as well as the conserved glycone and aglycone binding sites was performed on SmBgl1A deduced aminoacid sequence. The predicted secondary structure of Smbgl1 confirmed its appurtenance to GHI family: the presence of a classical (b/a)8 barrel and all the characteristic of subsite -1 (glycone site).

413. The transcriptional factors XYR1 and CRE1 regulate the expression of Cellulolytic and Xylanolytic genes at carbon source dependent-manner in *Hypocrea jecorina* (*Trichoderma reesei*). Amanda C.C. Antoniêto, Lílian S. Castro, Wellington R. Pedersoli, Roberto N. Silva. Department of Biochemistry and Immunology, School , University of São Paulo, Ribeirão Preto-SP, São Paulo, Brazil.

The ascomycete *Hypocrea jecorina* (anamorph of *Trichderma reesei*) is a one of the most well studied cellulolytic fungus and widely used in the biotechnology industry, such as in the production of second generation ethanol, because it is a strong producer of hydrolytic enzymes such as cellulases and xylanases. The objective of this study was evaluate the gene expression and enzymatic activity of cellulases and xylanases in the D*xyr1* and D*cre1* mutants and compare with the parental *T. reesei* (QM9414), in three different carbon sources. The strains were grown in Mandels-Andreotti medium, supplemented with cellulose, sophorose or glucose. The expression of 22 set cellulases and xylanases genes were evaluated by real-time PCR (qRT-PCR) and cellulolytic and xylanolytic activities were observed using different substrates. The *cel6a, cel3a, cel7b, cel3c, cel3e, xyn2* and *swo* genes showed a significantly high expression in the mutant D*cre1* when compared with the parental QM9414 and low expression of the *cel1a, cel3d* and *cel61b* genes was observed when compared the mutant D*xyr1* with the QM9414 on cellulose, sophorose and glucose. Overall, all of cellulase and xylanase genes showed higher expression in mutant D*cre1* and low expression in mutant D*xyr1* in all studied conditions, when compared to QM9414. Concerning to enzymatic profiles, the activity of CMCase, b-glucosidase and Xylanases ranged also for the presence of specific carbon source. These results suggest that the deletion of the genes xyr1 and cre1 affects the formation of cellulases and xylanases directly at transcriptional level and shown to be specific and dependent of the carbon source.

414. Characterization of tannic acid-inducible and hypoviral-regulated *CpsHsp*1 expression level of the chestnut blight fungus *Cryphonectria parasitica*. J.-H. Baek[1], J.-A. Park[1], J.-M. Kim[2], S.-M. Park[1], D.-H. Kim[1]. 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju, Chonbuk, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Chonbuk, South Korea.

A small heat shock protein gene, *CpsHsp*1, a ubiquitous chaperone in *Cryphonectria parasitica*, was characterized. The predicted protein sequence of *CpsHsp*1 gene contains a putative conserved domain, which is alpha crystallin domain (ACD) of alpha-crystallin-Hsps_p23-like superfamily. To characterize biological functions of the *CpsHsp*1 gene in the *C. parasitica*, the replacement vector for *CpsHsp*1-null mutant was designed to favor double crossover integration events. Disruption of the *CpsHsp*1 protein resulted in retarded growth rate, approximately 78.5% of the radial growth observed in the virus-free strain EP155/2. When the hypovirus CHV1 was transferred to the *CpsHsp*1-null mutant, all of the virus-containing *CpsHsp*1-null progeny displayed characteristics of invasive feeding hyphae, near absence of the typical mycelial mat on the surface, and sparse aerial hyphae. Northern blot analysis showed little accumulation of the *CpsHsp*1 gene transcript under normal growth conditions. However, the accumulation of the *CpsHsp*1 gene transcript was induced in modified Bavendamm's medium, which is a 0.7% tannic acid-.supplemented malt extract agar. To examine the viral regulation of the induction, the *CpsHsp*1 induction pattern in the isogenic hypovirulent strain UEP1 was compared with that in the wild-type strain EP155/2. Northern blot analysis of RNA from UEP1 cultured under induction conditions with tannic acid showed that hypoviral infection specifically reduced the level of *CpsHsp*1 transcript induced by tannic acid. To determine whether *CpsHsp*1 is induced by cool or heat stress, we additionally observed difference in the expression, and induction pattern of *CpsHsp*1 between virus-free EP155/2 and virus-infected hypovirulent UEP1 strains by Northern blot analysis and Western blot analysis.

415. Artificial miRNA constructs for *Phytophthora sojae* transformation. Stephanie R. Bollmann[1], Felipe D. Arredondo[1], Noah Fahlgren[2], James C. Carrington[2], Niklaus J. Grünwald[3], Brett M. Tyler[1]. 1) Center for Genome Research and Biocomputing, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Donald Danforth Plant Science Center, St. Louis, MO; 3) Horticultural Crops Research Laboratory, USDA Agricultural Research Service, Corvallis, OR.

Phytophthora, a genus of fungal-like oomycetes, contains some of the most devastating plant pathogens, causing multi-billion dollar damage to crops, ornamental plants, and natural environments. The genomes of five *Phytophthora* species, including the soybean pathogen *P. sojae*, have recently been sequenced, with many more species soon to be completed. Gene regulation by small RNA pathways is highly conserved among eukaryotes, although little is known about small RNA pathways in the Stramenopile kingdom. Two Dicer homologs, *DCL1* and *DCL2*, and one RDR homolog were cloned and annotated from *P. sojae*, and gene expression analysis revealed only minor changes in transcript levels among different lifestages and infection timepoints. At this point, the role of the two oomycete Dicer homologs are only speculated. This study aims to down-regulate *DCL1* and *DCL2* expression in order to analyze the contribution of each homolog to small RNA biogenesis. Traditional RNAi, such as overexpression of RNA complementary to a target mRNA transcript, has been used to knockdown gene expression in Phytophthora, although the effect is most often short-lived. Dicer homologs are involved in the RNAi pathway, therefore this method may not be effective, especially for the homolog involved in the siRNA pathway. Artificial miRNAs, designed from endogenous miRNAs, have recently been used to target transcripts such as these. We designed artificial miRNA constructs based on the conserved Phytophthora miRNA found in *P. sojae*, targeting both *DCL1* and *DCL2* as well as the effector *Avr1k*, the histidine biosynthesis enzyme *HISG*, and *GFP* for controls. Analysis of transformants is currently underway.

416. RNAi-dependent epimutations evolve antifungal drug resistance in the zygomycete fungal pathogen *Mucor*. Silvia Calo Varela[1], Cecelia Shertz[1], Robert J Bastidas[1], Soo Chan Lee[1], Piotr Mieczkowski[2], Joshua A Garnek[1], Rosa Ruiz-Vazquez[3], Santiago Torres-Martinez[3], Maria E Cardenas[1], Joseph Heitman[1]. 1) Molecular Genetics and Microbiol, DUKE University Medical Center, Durham, NC; 2) High Throughput Sequencing Facility, CCGS, UNC, Chapel Hill, NC; 3) Department of Molecular Genetics and Microbiology, University of Murcia, Murcia, Spain.

Microorganisms evolve via a panoply of mechanisms spanning aneuploidy, sexual/parasexual reproduction, mutators, Hsp90, and even prions. Mechanisms that may seem detrimental can be repurposed to generate diversity. The pathogenic fungus *Mucor circinelloides* grows as a hyphae aerobically, but as a yeast in anaerobic conditions or in the presence of the immunosuppressive drug FK506. FKBP12 is a protein folding enzyme conserved throughout eukaryotes that interacts with FK506 and mediates antifungal activity of this drug. The FK506-FKBP12 complex inhibits the protein phosphatase calcineurin and thereby blocks hyphal growth of *M. circinelloides*. Continued exposure to FK506 yields resistant isolates, which exhibit hyphal growth emerging from the yeast colony. Some isolates harbor a variety of mutations in the *fkbA* gene that encodes FKBP12. However, other isolates harbor no mutations in the *fkbA* gene. These unusual epimutant isolates also revert frequently within several generations of vegetative growth in drug-free media and are restored to wild-type (yeast growth in the presence of FK506). Northern and Western analyses revealed a loss of *fkbA* mRNA and FKBP12 protein in the epimutants. High-throughput sequencing and Northern blot also detected sRNA generated from *fkbA* in the epimutant strains, revealing a new role for RNAi in the development of transient, reversible resistance to an antifungal drug treatment. RNAi could be triggered via dsRNA production from an overlap in the 3' regions of the mRNA of *fkbA* and its neighboring gene *patA*, which encodes a putative polyamine transporter. Our results reveal a novel epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity in fungi.

417. Heterochromatic marks are involved in the repression of plant-regulated secondary metabolism in *Epichloë festucae* and for symbiotic interaction with the host perennial ryegrass. Tetsuya Chujo, Barry Scott. Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.

The fungal endophyte *Epichloë festucae* systemically colonizes perennial ryegrass (*Lolium perenne*), and produces a range of secondary metabolites to protect the host plant. The alkaloid peramine provides protection against insect herbivory. Protection against mammalian herbivory is afforded by the production of alkaloids, such as ergot alkaloids and lolitrems. Using the *E. festucae*-perennial ryegrass symbiotic association as a model experimental system we have shown that gene clusters for the synthesis of these bioprotective metabolites are all preferentially and highly expressed *in planta*, but not expressed in culture. Recent work showed that disruption of genes encoding either heterochromatin protein-1 (HepA) or the H3K9 methyltransferase (ClrD) in *Aspergillus nidulans* resulted in enhanced expression of secondary metabolite gene clusters, demonstrating that heterochromatic marks are involved in the repression of these clusters. Thus, we hypothesized that plant-regulated *E. festucae* secondary metabolite gene clusters have a repressive chromatin structure in culture, and chromatin remodeling is an important component for activation of these gene clusters *in planta*. To test this hypothesis we have deleted the *hepA* and *clrD* homologues from *E. festucae* by targeted gene replacement. Deletion of *hepA* resulted in a slight reduction in culture radial growth whereas deletion of *clrD* resulted in a severe reduction. Western blot analysis revealed that the level of H3K9 tri-methylation (H3K9me3) is dramatically decreased in *DclrD* mutants. Expression levels of *ltmG* & *ltmM* (cluster 1) and *ltmP* & *ltmF* (cluster 2), as measured by qRT-PCR, increased in both the *DhepA* and *DclrD* mutants grown in a defined medium. Introduction of a wild-type allele of either *hepA* or *clrD* complemented *DhepA* or *DclrD* mutant phenotypes, respectively. In addition, the *DhepA* mutant has a dramatic host interaction phenotype, inducing severe stunting and premature senescence of the ryegrass host. On the other hand, *DclrD* mutant is an infection mutant. These results strongly suggest that heterochromatic marks regulate both secondary metabolite gene expression and the mutualistic symbiotic interaction of *E. festucae* with its host perennial ryegrass.

418. Cellulose Degradation Regulator 2 Induces Expression of a Conserved Core of Genes for Plant Cell Wall Saccharification in *Neurospora crassa* and *Aspergillus nidulans*. Samuel T. Coradetti, Yi Xiong, N Louise Glass. Department of Plant and Micorbial Biology, University of California, Berkeley, CA.

To better understand mechanisms of cellulase gene regulation and genome-wide gene regulation enabling robust enzyme secretion, we studied the conservation of gene regulation by cellulose degradation regulator 2 (CLR-2) in *Neurospora crassa* and *Aspergillus nidulans*. Misexpression of CLR-2 under normally repressive and non-inducing culture conditions was sufficient for cellulases secretion in *N. crassa*, but not *A. nidulans*. We used RNAseq to map the trascriptome in wild-type, deletion and mis-expression strains of both species. We identified a cohort of conserved enzymes with conserved sequence and CLR-2 dependent regulation across evolutionarily divergent ascomycetes, which represent a core of essential enzymes for degradation of complex cellulosic substrates. We also identified non-conserved CLR-2 regulated genes in each species, which may have function specific to a particular substrate or niche. These data suggest that manipulation of CLR-2 has significant potential for improved cellulase production from industrial production strains.

419. The transcriptional repressor CRE-1 regulates glycogen metabolism in *Neurospora crassa*. Fernanda B. Cupertino, Stela Virgilio, Fernanda Z. Freitas, Thiago S. Candido, Maria Célia Bertolini. Instituto de Quimica,UNESP, Araraquara, São Paulo, Brazil.

In *Neurospora crassa* the RCO-1 co-repressor, an orthologue of the yeast Tup1, has been identified as a protein involved in glycogen metabolism regulation in a screening of a transcription factor knocked-out strains set. The *Saccharomyces cerevisiae* Tup1 protein participates in the Tup-1-Ssn6

complex, which represses the expression of genes through its interaction with other protein partners, such as the yeast Mig1 repressor, mediator of carbon catabolite repression (CCR). The *N. crassa* CRE-1 is an orthologue of Mig1p and has been reported to regulate a number of genes by binding to the CreA motif 5'-SYGGRG-3'. In this work we investigated if CRE-1 transcription factor regulates glycogen metabolism and whether depends on the RCO-1/RCM-1 complex, the ortologue of the yeast Tup-1/Ssn6. First, we demonstrated that CRE-1 is involved in catabolic repression in *N. crassa*. Growth of the wild-type strain in VM medium containing xylose + 1 mM 2-deoxyglucose (2-DG) was drastically reduced, while growth in the *cre-1*^{KO} was not affected by 2-DG. The *cre-1*^{KO}, *rco-1*^{KO} and *rcm-1*^{RIP} mutant strains showed impaired glycogen accumulation when compared to the wild-type strain, being the higher levels observed in the *cre-1*^{KO} strain. Glycogen accumulated by this mutant strain was much higher than the wild-type strain under both repressed (glucose) and non-repressed (xylose) carbon source, suggesting that the carbon source has no influence in the glycogen accumulated. The DNA motif 5'-SYGGRG-3' was identified in the promoters of genes encoding for synthesis (*gnn*, *gsn*, and *gbn*) and degradation enzymes (*gpn*, *gdn*). Binding of the GST::CRE-1 recombinant protein to the CRE-1 motifs in the *gsn* and *gpn* promoters was confirmed by DNA gel shift, indicating that both genes must be regulated by CRE-1. Gene expression was analyzed by qRT-PCR and all genes were differently expressed in the mutant strains, and for some genes, gene expression correlated well with the levels of glycogen accumulated. ChIP-PCR assay was performed to confirm in vivo CRE-1 binding to the promoters and the results suggested that the CRE-1, RCO-1 and RCM-1 proteins likely interacted. All results together indicated that the CRE-1 transcription factor acts as a repressor in glycogen regulation, and might require the interaction with RCO-1 and RCM-1 co-repressors. Supported by FAPESP and CNPq.

420. Transcriptional Response to Hypoxia in the Dimorphic Fungus *Histoplasma capsulatum* . Juwen C. DuBois[1,3], A. George Smulian[2,3]. 1) Pathobiology and Molecular Medicine Graduate Program, University of Cincinnati, Cincinnati, OH; 2) Infectious Disease Division, University of Cincinnati, Cincinnati, OH; 3) Cincinnati VA Medical Center, Cincinnati OH.

Background and purpose: The incidence of life-threatening fungal infections has increased because of a rapidly growing population of immunocompromised individuals. *Histoplasma capsulatum* (*Hc*) is a thermally dimorphic fungal pathogen which causes pulmonary and systemic disease in both immunocompetent and immunocompromised individuals. All fungal pathogens encounter microenvironmental stresses as they colonize and infect the mammalian host and their ability to adapt to environmental changes is critical for pathogenicity. While oxygen is essential for the survival of most eukaryotic organisms, low oxygen availability or hypoxia is one microenvironmental stress that is known to occur during infection. Therefore, we aim to determine the mechanism by which *Hc* is able to survive and adapt to hypoxia by determining the significance of its transcriptional response to hypoxia. **Methods:** *Hc* yeast cells were cultured to mid-log phase then transferred to normoxic (~21%) or hypoxic conditions (<1% O2). After culture under these conditions, *Hc* viability was determined and RNA extracted. RNA was subjected to high throughput RNA sequencing (RNA-seq) and quantitative real-time PCR analysis of the predicted upregulated genes, *Hc*SREBP and *Hc*UPC2. Selected key genes were downregulated using RNAi-mediated silencing and the viability of *Hc* under hypoxia in the absence of expression of these genes was assessed. **Results:** After exposure to hypoxia, *Hc* viability remained between 80-94% through all time points. RNA-seq analysis demonstrated a significant increase in expression of NIT50, a swirm domain protein-DNA binding protein, a predicted ABC transporter, an NADPH oxidoreductase and the RSP guanine nucleotide exchange factor. Although RNA-seq analysis did not reveal significant increases in SREBP and UPC2, these genes are known hypoxia responsive transcriptional regulators in fungi. Computational transcription factor binding site analysis revealed predicted human SREBP and *Aspergillus fumigatus* SrbA binding sites upstream of all genes upregulated under hypoxic conditions. RNAi-mediated silencing of *Hc*SREBP and *Hc*UPC2 gene expression impaired the ability of Hc to grow under hypoxia. **Conclusion:** Creation of *Hc* strains with impaired survival under hypoxia allows characterization of the role of hypoxic adaptation to *Hc* intracellular survival.

421. The *Neurospora crassa* SEB1 transcription factor binds to STRE motif and modulates stress responses through different pathways. F. Freitas, F. Cupertino, R. Gonçalves, M. C. Bertolini. Instituto de Química-UNESP, Araraquara, São Paulo State, Brazil.

Glycogen is a polysaccharide widely distributed in microorganisms and animal cells. Its metabolism is highly conserved in eukaryotes, being glycogen synthase (GSN) and glycogen phosphorylase (GPN) the rate-limiting enzymes in the synthesis and degradation, respectively. Previous studies demonstrated that the down-regulation of the gene encoding GSN (*gsn*) under heat shock might require the two STress Response Element (STRE) existent in its 5'-flanking region. In *Saccharomyces cerevisiae* the Msn2/4p transcription factors bind to STRE regulating expression of genes under different stress conditions. However orthologues of Msn2/4p were not identified in *N. crassa*. The ORF NCU02671 product (named here as SEB1) was identified in pull-down assays and mass spectrometry as a protein able to bind to STRE. This protein has two C_2H_2 Zn-finger DNA binding domains and shares 40% and 32% identity with, respectively, the *Trichoderma atroviridae* Seb1 and *Aspergillus fumigatus* SebA transcription factors. While Seb1 is the functional orthologue of Msn2p/4p, SebA plays a more complex role, participating in several stress tolerance pathways, such as heat shock and oxidative stresses. *N. crassa* GST::SEB1 bound specifically *in vitro* to both STRE motifs of the *gsn* promoter as demonstrated by EMSA. The *seb1*^{KO} mutant strain accumulated higher levels of glycogen than the wild-type strain under vegetative growth (30ºC) and the glycogen content was drastically reduced after heat stress (45ºC). The *gsn* and *gpn* gene expression at 30ºC was decreased in the mutant strain as compared to the wild-type strain. However at 45ºC *gpn* expression in the mutant strain was much higher than that in the wild-type strain, what may explain the reduced levels of glycogen in the mutant strain under the same temperature. Growth of the *seb1*^{KO} mutant strain under different stress conditions showed that SEB1 is involved in heat, osmotic and oxidative stresses. The mutant strain was unable to growth at 45ºC and growth was recovered when the culture was shifted back to 30ºC. Moreover, the mutant strain displayed defective growth in the presence of NaCl, sorbitol, and paraquat but not in the presence of hydrogen peroxide, suggesting that SEB1 controls gene expression under different stress conditions acting through different signaling pathways. Supported by FAPESP, CNPq, and CAPES.

422. WITHDRAWN

423. Regulation of glycolysis and gluconeogenesis by antisense transcription in *Aspergillus nidulans*? Michael Hynes[1], Koon Ho Wong[2], Sandra Murray[1]. 1) Dept Gen, Univ Melbourne, Parkville, Victoria, Australia; 2) Dept. of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA.

The last step in glycolysis is carried out by pyruvate kinase, encoded by *pkiA*, converting phospho-enol-pyruvate to pyruvate for metabolism to oxaloacetate and acetyl-CoA. The key step in gluconeogenesis is conversion of oxaloacetate to phospho-enol-pyruvate by PEP carboxykinase, encoded by *acuF*. Simultaneous activity of these enzymes would generate a nasty futile cycle. A number of observations suggests that control of the expression of these two genes involves activation of sense transcription and negative control by activation of antisense transcription. For *pkiA*, ChIP studies have found binding of the gluconeogenic activators AcuK and AcuM and of the acetate dependent FacB activator in the downstream region. Cognate binding sites are conserved in filamentous ascomycetes. RNA Seq, polII ChIP and RT-PCR analysis indicates antisense transcription during growth on acetate or proline as carbon sources. Old data (de Graaf, van den Broek, Visser; Cur. Genetics 13: 315, 1988) showed that transformation of a construct lacking these 3' sites resulted in inappropriate *pkiA* expression on acetate. In response to growth on gluconeogenic carbon sources, the *acuF* gene is activated by AcuK and

AcuM binding to sites in the 5' upstream region. Studies with an *acuF-lacZ* gene fusion indicate positive control by AcuK and AcuM but a loss of the glucose repression observed in Northerns suggesting negative regulation acting via 3' sequences in response to growth on glycolytic carbon sources. Support for this is provided by transcription studies. Modulation of the balance between the opposing activities of these two gene products is proposed to result from transcriptional interference involving collision of RNA polymerase molecules.

424. ClbR and its paralog, ClbR2, regulate gene expression of cellulase genes in response to cellobiose in *Aspergillus aculeatus*. E. Kunitake, S. Tani, J. Sumitani, T. Kawaguchi. Life & Environmental Science, Osaka Prefecture University, Sakai, Osaka, Japan.

The cellobiose- and cellulose-responsive induction of the cellobiohydrolase I (*cbhI*) and carboxymethylcellulase 2 (*cmc2*) genes is not regulated by XlnR, a $Zn(II)_2Cys_6$ transcriptional activator, in *Aspergillus aculeatus*. We have identified a novel activator containing a $Zn(II)_2Cys_6$ binuclear cluster motif designated as cellobiose-response regulator (ClbR), and which is not homologous to Clr-2/ClrB, a transcriptional activator controlling cellobiose-responsive induction in *Neurospora crassa* and *Aspergillus nidulans*. Interestingly ClbR regulates not only the expression of *cbhI* and *cmc2* but also genes regulated by XlnR under the cellulose-inducing condition. However, the *clbR* overexpression did not increase the expression of all genes under control of ClbR. Therefore, we predict that ClbR functions cooperatively with other factor(s). This study reports the ClbR function and the functional relationships among ClbR, ClbR-interacting factor, and ClrB homolog on cellobiose-responsive induction in *A. aculeatus*. ClbR-interacting proteins were screened from a prey library composed of ClbR paralogs and transcription factors controlling the expression of glycoside hydrolase genes by yeast two-hybrid method. ClbR2, 42% identity to ClbR, was so far isolated as a ClbR interacting protein. To investigate the correlation between ClbR and ClbR2 function, gene expression profiles under control of ClbR were assessed in the *clbR* amd *clbR2* single disruption mutants, and *clbR*/*clbR2* double disruption mutant. The expression of *cbhI* and *cmc2* decreased to the same level in all three mutants under the cellobiose-inducing condition. Furthermore, transcripts of the *cbhI* and *cmc2* genes drastically decreased in the *clrB* disruption mutant. This result suggests that ClrB regulates gene expression controlled via the XlnR-independent signaling pathway in this fungus. Because the *clrB* gene is induced by cellulosic compounds, we investigated if *clrB* expression is regulated by ClbR and ClbR2. In the *clbR*, *clbR2*, and *clbR*/*clbR2* mutants, *clrB* transcripts under the inducing condition reduced to almost the same level as those under the uninducing condition. Taken together, these data demonstrate that ClbR and ClbR2 regulate the cellulase gene expression in response to cellobiose by regulating the *clrB* gene expression.

425. Expression of a bacterial xylanase in *Trichoderma reesei* under the *egl2* and *cbh2* glycosyl hydrolase gene promoters. Helena Nevalainen[1,2], Shingo Miyauchi[1,2], Junior Te'o[1,2], Peter Bergquist[1,2,3]. 1) Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia; 2) Biomolecular Frontiers Research Centre, Macquarie University, NSW 2109, Australia; 3) Department of Molecular Medicine & Pathology, University of Auckland Medical School, Auckland 1142, New Zealand.

Our main aim in this study was to isolate a suite of promoters, other than *cbh1*, to establish a gene expression platform in which they could be used synergistically for the expression of recombinant gene products, using a bacterial thermophilic xylanase (XynB) as an example. The *egl2* and *cbh2* promoters were selected on the basis of their relative strength and functionality under the same cultivation conditions that induce *cbh1* so that this promoter, widely used for recombinant expression, could be included in the 'promoter mix' when desired in the future. Together, these promoters possess considerable expression capacity that can be harnessed for the synthesis of recombinant gene products in *T. reesei*. We also explored the mode of glycosylation of the recombinant bacterial xylanase in *T. reesei* to confirm that the higher molecular weight bands seen in activity zymograms were indeed post-translationally modified by glycosylation and to characterize the sugars attached to the protein. The highest XynB production was achieved from a transformant containing 1-2 copies of the EGL2sigpro vector (*egl2* promoter). Best xylanase producers did not show any particular pattern in terms of the number of gene copies and their mode of integration into the chromosomal DNA. Transformants produced multiple forms of XynB which were decorated with various N- and O-glycans. One of the O-glycans was identified as hexuronic acid, whose presence had not been observed previously in the glycosylation patterns of *T. reesei*.

426. A single *argonaute* gene participates in exogenous and endogenous RNAi and controls different cellular functions in the basal fungus *Mucor circinelloides*. F. E. Nicolas-Molina[1,2], M. Cervantes[1], A. Vila[1], S. Moxon[3], J. P. De Haro[1], T. Dalmay[3], S. Torres-Martínez[1], R. M. Ruiz-Vázquez[1]. 1) Department of Genetics and Microbiology, University of Murcia, Murcia, Spain; 2) Regional Campus of International Excellence "Campus Mare Nostrum", Murcia, Spain; 3) School of Biological Sciences, University of East Anglia, Norwich, UK.

Regulation by RNAi of diverse cellular functions in metazoans is largely known. However, although different classes of endogenous small RNAs (esRNAs) have been identified in fungi, their biological roles are poorly described. The Argonaute proteins are the core component of all known RNAi pathways. Here we identified three *argonaute* genes of the basal fungus *Mucor circinelloides* and investigated their participation in exogenous and endogenous RNAi. Only *ago-1* is required for transgene-induced RNA silencing. Ago-1 is also required for the production of distinct classes of esRNAs derived from exons (ex-siRNAs), which differ in the silencing proteins required for their biogenesis. Classes I and II ex-siRNAs bind to Ago-1 to control mRNA accumulation of the target protein coding genes. Class III ex-siRNAs do not specifically bind to Ago-1, but require this protein for their production, revealing the complexity of the biogenesis pathways of ex-siRNAs. We also show that *ago-1* gene is involved in the response to environmental signals, since vegetative development and autolysis induced by nutritional stress are affected in *ago-1*$^-$ mutants. Our results highlight the role of ex-siRNAs in the regulation of endogenous genes in basal fungi and expand the range of biological functions modulated by RNAi silencing. This work was funded by MICINN (BFU2009-07220) and MINECO (BFU2012-32246), Spain.

427. The transcription factor, AtrR, regulates the expression of ABC transporter genes and ergosterol biosynthesis genes in aspergilli. Ayumi Ohba[1], Kiminori Shimizu[2], Daisuke Hagiwara[2], Takahiro Shintani[1], Susumu Kawamoto[2], Katsuya Gomi[1]. 1) Div. Biosci. Biotechnol. Future Bioind., Grad Sch. Agric. Sci., Tohoku Univ., Japan; 2) MMRC, Chiba Univ., Japan.

We previously demonstrated that a novel Zn(II)2Cys6 transcriptional factor, AoAtrR, regulates gene expression of the ABC transporters that would function as drug efflux pumps and contributes to the azole resistance in *Aspergillus oryzae*. Moreover, we showed that a deletion mutant of the *AoatrR* ortholog (*AfatrR*) in *Aspergillus fumigatus* was similarly hypersensitive to azole drugs. However, little is known about target genes regulated by AfAtrR. In this study, we comprehensively examined the target genes regulated by AfAtrR using next-generation DNA sequencing technology (RNA-seq). RNA-seq analysis indicated that AfAtrR similarly regulated at least one ABC transporter. In addition, surprisingly, AfAtrR also regulated several ergosterol biosynthetic pathway genes including *erg11*(*cyp51A*). It has been known that the basic helix-loop-helix transcription factor, SrbA, has a critical role in ergosterol biosynthesis and resistance to the azole drugs in *A. fumigatus*. Interestingly, the ergosterol biosynthetic pathway genes regulated by AfSrbA were nearly identical with those regulated by AfAtrR. Therefore, we investigated difference in function between AtrR and SrbA in *A. oryzae*. The expression of ergosterol biosynthetic pathway genes such as *erg11*, *erg24*, and *erg25* etc. and three ABC transporter genes was significantly down-regulated in the

disruption mutant of *AoatrR*. Similarly, the expression of same ergosterol biosynthetic pathway genes was also markedly down-regulated in the disruption mutant of *AosrbA*, but the expression of ABC transporter genes was not affected. In contrast, the expression of ergosterol biosynthetic pathway genes was not up-regulated in an overexpression strain of *AoatrR*. These results suggest that AtrR and SrbA coordinately regulate ergosterol biosynthetic pathway genes in aspergilli. On the other hand, the *AoatrR* disruptant was more hypersensitive to azole drugs compared to the *AosrbA* disruptant, suggesting that hypersensitivity of the *atrR* disruptant to azole drugs is attributed not only to lowered ergosterol levels due to down-regulation of ergosterol biosynthetic pathway genes, but also to reduced efflux transport of the drugs due to down-regulation of ABC transporter genes.

428. Effects of intron deletions in production of a heterologous protease in *Trichoderma reesei*. Marja Paloheimo. Roal Oy, Rajamäki, Finland.

A protease gene originating from a filamentous fungus *Malbranchea cinnamomea* was expressed in *Trichoderma reesei* using the strong *T. reesei cbh1* (*cel7A*) promoter. The heterologous protease was produced at relatively high yields. However, when the protease cDNA was included in the (otherwise identical) expression cassette the amount of protease in the culture supernatants of the transformants was very low. The native *M. cinnamomea* protease gene contains three introns. To further study the effects of the removal of introns in the production of protease, single-copy transformants were constructed that contained expression cassettes in which each of the three introns was separately removed and in which two of the introns (all combinations) were deleted at a time from the genomic protease gene. The expression cassettes were targeted to the native *cbh1* locus. Three single copy transformants from each transformation were chosen for further studies. These strains were cultivated in shake flasks and the amount of protease was analysed from the culture supernatants. A clear decrease in the protease activity was detected when any of the introns was removed. However, depending on the intron removed there were differences in the relative decreases of protease activity. Removal of two introns at a time had a cumulative decreasing effect on production of protease. The results obtained are shown and discussed.

429. Novel core promoter elements in the oomycete *Phytophthora infestans* and their influence on expression pattern detected by genome-wide analysis. Laetitia Poidevin, Sourav Roy, Howard S. Judelson. Department of Plant Pathology & Microbiology, University of California Riverside, USA.

The core promoter is the region flanking the transcription start site (TSS) that directs pre-initiation complex formation. While core promoters have been studied intensively in mammals and yeast, little is known about more diverse eukaryotes including oomycetes. Prior studies of a small collection of cloned oomycete genes proposed that its core promoters contain a 19-nt block bearing both an Initiator-like sequence (INR) and a novel 3' sequence named FPR, but this has not been extended to whole-genome analysis. To learn more about oomycete core promoters, we used expectation maximization to find over-represented motifs near TSSs of *Phytophthora infestans*, the potato blight pathogen. The motifs corresponded to INR, FPR, and a new element found 25-nt downstream of the TSS called DPEP. TATA boxes were not detected. Assays of DPEP function by mutagenesis were consistent with its role as a core motif. Genome-wide searches found a well-conserved combined INR+FPR in only 13% of genes after correcting for false discovery, contradicting prior reports that INR and FPR are adjacent to each other in most genes. INR or FPR were found alone near TSSs in 18% and 7% of genes, respectively. Promoters lacking the motifs had pyrimidine-rich regions near the TSS. The combined INR+FPR motif was linked to higher than average mRNA levels, developmentally-regulated transcription, and functions related to plant infection, while DPEP and FPR were over-represented in constitutive housekeeping genes. The motifs were all detected in other oomycetes including *Hyaloperonospora arabidopsidis*, *Phytophthora sojae*, *Pythium ultimum*, and *Saprolegnia parasitica*, but only INR seemed present in a non-oomycete stramenopile. The absence of a TATA box and presence of novel motifs show that oomycete core promoters have diverged from that of previously-studied model systems, and likely explains failures in prior heterologous expression studies. The association of the INR+FPR with developmentally-regulated genes shows that oomycete core elements influence stage-specific transcription in addition to regulating pre-initiation complex formation.

430. New insights into the phosphate-sensing network in *Neurospora crassa*. Antonio Rossi[1], Gabriela F Persinoti[2], Nalu T A Peres[2], Diana E Gras[2], Nilce M Martinez-Rossi[2]. 1) Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, SP, Brazil; 2) Genética, FMRP-USP, Ribeirão Preto, SP, Brazil.

The filamentous fungus *Neurospora crassa* is an excellent model system for examining molecular responses to ambient signals in eukaryotic microorganisms. Inorganic phosphate is an essential growth-limiting nutrient in nature and is crucial for the synthesis of nucleic acids and the flow of genetic information. Numerous ambient signals activate the recruitment of mitogen-activated protein kinase (MAPK) cascades. Thus, to identify genes involved in metabolic responses to exogenous phosphate sensing and the functioning of an MAPK, MAK-2, we performed microarray experiments using a *mak-2* knockout strain (*Dmak-2*) grown under phosphate-shortage conditions by comparing its transcription profile to that of a control strain grown in low- and high-phosphate cultures. These experiments revealed 912 unique differentially expressed genes involved in a number of physiological processes related to phosphate transport, metabolism, and regulation as well as posttranslational modification of proteins, and MAPK signaling pathways. Quantitative real-time-PCR gene expression analysis using independent RNA samples of 10 arbitrarily chosen genes validated our microarray results. A high Pearson correlation between microarray and quantitative real-time-PCR data was observed. The analysis of these differentially expressed genes in the *Dmak-2* strain provide evidence that the *mak-2* gene is a component of the hierarchical phosphate-signaling pathway in *N. crassa* in addition to its independent involvement in other metabolic routes such as the isoprenylation pathway. In this extended model, MAK-2 is functional regarding transcription of Pi-repressible phosphatases when *N. crassa* is cultured under Pi shortage and is non-functional under abundant Pi conditions, thus revealing novel aspects of the *N. crassa* phosphorus-sensing network. Financial support: FAPESP, CNPq, CAPES, FAEPA.

431. Carbon source and light dependent regulation of gene clusters in *Trichoderma reesei* (*Hypocrea jecorina*). Doris Tisch[2], Monika Schmoll[1]. 1) Health and Environment, Bioresources, Austrian Institute of Technology AIT, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria.

Trichoderma reesei (anamorph of Hypocrea jecorina) is one of the most prolific producers of plant cell wall degrading enzymes. Regulation of the genes encoding these enzymes occurs in response to the nutrient sources available in the environment and many of them are responsive to light as well. Cellulose as the natural substrate induces the most complete enzyme set, while induction of cellulases also occurs on sophorose and lactose. In contrast, no cellulases are induced on glycerol and the respective genes are repressed on glucose. We therefore investigated the transcriptome on these five carbon sources in light and darkness and aimed to identify genes specifically expressed under cellulase inducing conditions. These conditions are characterized by a significant enrichment of genes involved in C-compound and carbohydrate degradation and transport among the upregulated gene set. Genes down-regulated under inducing conditions show a significant enrichment in amino acid metabolism and energy metabolism. We were further interested whether light dependent regulation is clustered in the genome and if the carbon source is relevant for activation of light dependent clusters. We found that light dependent clustering predominantly occurs upon growth on cellulose, with the most significant regulation in a gene cluster comprising env1. This cluster appears on glucose as well, but is not down regulated in mutants of blr1 or blr2. Also cbh2, the arabinofuranosidase gene abf2 and the histone acetyltransferase gene gcn5 are part of light dependent clusters. Hierarchical clustering of gene expression patterns was performed to reveal functional

divergence of gene regulation with respect to light response or carbon specific regulation. Glycoside hydrolase genes follow the whole transcriptome pattern with carbon source being superior to light in terms of regulation. ENV1 in in part the G-protein beta subunit GNB1 were found to be crucial for carbon source specific regulation of G-protein coupled receptors, genes involved in secretion, sulphur metabolism and oxidative processes as well as transporters. We conclude that clustered regulation of light responsive genes preferentially occurs upon growth cellulose and that ENV1 and to a lesser extent GNB1 play a role in carbon source dependent regulation of specific gene groups in light.

432. Identification of regulators for enzyme production in Trichoderma reesei using genome-wide approaches. M. Häkkinen, M. Valkonen, N. Aro, M. Vitikainen, A. Westerholm-Parvinen, M. Penttilä, M. Saloheimo, T. Pakula. VTT, Espoo, Finland.

Trichoderma reesei (anamorph *Hypocrea jecorina*) is an efficient producer of enzymes degrading cellulosic and hemicellulosic biomass. The cellulases and hemicellulases produced by the fungus are widely employed in industry, and also in biorefinery applications. Various environmental and metabolic factors together with the physiological state of the cell affect the enzyme production of *T. reesei*. Thus, a complex signalling cascade and regulatory network is needed for the accurate timing of hydrolytic enzyme production and to control the pattern of enzyme activities produced. In previous studies, both positively and negatively acting regulatory factors for cellulase and hemicellulase genes have been characterised in *T.reesei*. In this study, an expression microarray data on *T. reesei* cultivated in the presence of different carbon sources was analysed in order to identify additional regulatory genes for cellulase and hemicellulase production. In total, 28 putative regulatory factors for *T. reesei* cellulases and hemicellulases were identified and selected for further studies. The genes were overexpressed in *T. reesei* QM9414. Cultivated modified strains were tested for their ability to produce cellulases, xylanases and total secreted protein. Over-expression of seven of the genes led to increased production of cellulases and/or xylanases.

433. The central core of the response to light and injury and their regulation by RNAi machinery in the filamentous fungus *Trichoderma atriviride*. J.M. Villalobos-Escobedo, N. Carreras-Villaseñor, A. Herrera-Estrella. LANGEBIO-CINVESTAV, Irapuato, Guanajuato, Mexico.

All living organisms must sense and respond appropriately to different environmental stimuli in order to survive. *Trichoderma atroviride* is a filamentous fungus with wide adaptability to different environmental conditions and is considered a good morphogenetic model because it respond to light and injury producing asexual reproductive structures (conidia). The mechanisms used by this fungus to respond to these stimuli have been studied independently and models of perception and signal transduction for each stimulus have been proposed. In our research group, we found that the *Ddcr2* mutant, involved in small RNA biogenesis, is affected in conidiation in response to light and injury, indicating that conidiation is regulated by small RNAs. In this work we discovered that when the *Ddcr2* mutant receives simultaneously light and injury, it is able to conidiate. Based on this observation, we propose that there is a central core of genes needed to coordinate the response to both stimuli and in the *Ddcr2* the signaling pathways act synergistically to achieve the correct expression of these genes in order to conidiate. To test the above hypothesis we analyzed the transcriptome in response to light and injury of the wild type (WT), and identified 38 genes that have the same expression profile in response to the two stimuli, 19 of them are induced and 19 are repressed, called the central core genes, most of the up-regulated genes are involved in RNA processing, ribosome biogenesis, chromatin remodeling and other cellular processes indicating that this core regulates gene expression to respond to the stimulus. While genes that are repressed are mainly involved in lipid, carbohydrate and protein metabolic processes suggesting that a metabolic arrest is necessary to respond to both stresses. The expression analysis of this core of genes in the *Ddcr2* revealed some of them are deregulated, but other genes respond similarly to WT in either light or injury in *Ddcr2*, so this group of genes is regulated by the RNAi machinery.

434. RNA-mediated Gene Silencing in Candida albicans: Reduction of Fungal Pathogenesis by Use of RNAi Technology. M. Moazeni[1], MR. Khorramizadeh[2], P. Kordbacheh[1], H. Zeraati[3], F. Noorbakhsh[4], L. Teimoori-Toolabi[5], S. Rezaie[1,2]. 1) Dept. of Medical Mycology & Parasitology, Tehran University of Medical Sciences, Tehran, Iran; 2) Dept. of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran; 3) Dep. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 4) Dept of Biology, Islamic Azad University, Varamin-Pishva Branch, Varamin, Iran; 5) Dept of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Iran.

Background: The introduction of RNA silencing machinery in fungi has led to the promising application of RNAi methodology to knock down essential vital factor or virulence factor genes in the microorganisms. Efg1p is required for development of a true hyphal growth form which is known to be essential for interactions with human host cells and for the yeast's pathogenesis. In addition, it is responsible for positive regulation of the expression of several hyphal-specific genes: SAP5, which encodes secreted aspartic proteinase, and ALS3, which encodes a multi-functional adhesive polypeptide. In this paper, we describe the development of a system for presenting and studying the RNAi function on the EFG1 gene in Candida albicans. Materials and Methods: The 19-nucleotide siRNA was designed on the basis of the cDNA sequence of the EFG1 gene in Candida albicans and transfection was performed by use of a modified-PEG/LiAc method. To investigate EFG1 gene silencing in siRNA-treated cells, the yeasts were grown in human serum; to induce germ tubes a solid medium was used with the serum. Quantitative changes in expression of the EFG1 gene as well as two Efg1-associated genes, ALS3 and SAP5, were analyzed by measuring the cognate EFG1, SAP3 and ALS3 mRNA levels by use of a quantitative real-time RT-PCR assay. Results: Images taken by fluorescent microscopy method indicated the effectiveness of transfection. Compared with the positive control, true hyphae formation was significantly reduced by siRNA at concentrations of 1 mM, 500 nM, and 100 nM (P227.05). According to REST® software data analysis, a considerable decrease in EFG1 gene expression was observed when applying both 500 nM and 1mM of siRNA ($P<0.05$). Consequently, the expression of ALS3 and SAP5 were significantly down-regulated both in yeast treated with 500 and 1 mM of siRNA ($r<0.05$). Conclusion: On the basis of the potential of post-transcriptional gene silencing to control the expression of specific genes, these techniques may be regarded as promising means of drug discovery, with applications in biomedicine and functional genomics analysis.

435. RNAi machinery controls the asexual reproduction induced by light of the filamentous fungus *Trichoderma atroviride*. N. Carreras-Villaseñor[1], EU. Esquivel-Naranjo[2], JM. Villalobos-Escobedo[1], C. Abreu-Goodger[1], A. Herrera-Estrella[1]. 1) LANGEBIO-Cinvestav, Irapuato, Guanajuato, Mexico; 2) Facultad de Ciencias Naturales. Universidad Autónoma de Queretaro, Mexico.

RNA silencing is a mechanism through which sRNAs induce the inactivation of genes by mRNA degradation, translation inhibition, chromatin remodeling, and DNA elimination. Dicer, Argonaute and RNA dependent RNA Polymerase (RdRP) are the RNAi components, and participate in the biogenesis and function of most sRNAs. Representatives of the kingdom Fungi possess multiple RNA silencing components in their genome. However, the physiological roles of the RNAi pathway in fungi have been poorly studied. Here, we report the phenotypical and transcriptional analysis in RNAi machinery mutants in the filamentous fungus *Trichoderma atroviride*. We found a defect in conidiation induced by light in the D*dcr2*, D*dcr1*D*dcr2*, D*rdr3* and D*ago2*. Light responsive genes are different among the dicer mutants and WT, therefore the processes that are turned on and off in each strain are different too. Genes involved in metabolism, chromatin remodeling and growth are differentially expressed in the mutants compared with the WT, which denote the possible

participation of the RNAi machinery in these processes. In addition, different classes of sRNAs are differentially expressed between the WT and D*dcr2* strains. An in depth analysis of features of these small RNAs, e.g. size, type of sRNAs and 5'nucleotide bias, show that those present in WT are lost in the sRNAs of the D*dcr2* strain. All together these data show that in *Trichoderma atroviride*, the RNAi machinery has a central role in endogenous processes such as the development and fitness, beyond controlling the protection of genome against invasive nucleic acids as reported for other fungi.

436. Genome-wide analysis of light responses in *Mucor circinelloides*. Victoriano Garre, Sergio López-García, Eusebio Navarro, Santiago Torres-Martínez. Departamento de Genética y Microbiología, Universidad de Murcia, 30100 Murcia, Spain.

Light regulates developmental and physiological processes in a wide range of fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The zygomycete *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available. *Mucor*, like other Zygomycetes, has three *white collar-1* genes (*mcwc-1a, mcwc-1b* and *mcwc-1c*) that code for proteins which present characteristics of photoreceptors. Each *mcwc-1* gene controls a specific response to light. Thus, *mcwc-1a* and *mcwc-1c* control phototropism and photocarotenogenesis, respectively, whereas the *mcwc-1b* function in regulation by light has not been proved. In order to deepen in the regulation by light in *Mucor*, a systematic approach using microarrays was followed to characterize white light-inducible transcriptional changes in wild-type and knockout mutants for each *mcwc-1* gene. Analysis of microarray data revealed that light is mainly a positive signal for transcription in *Mucor*, as in other fungi, since 123 genes were up-regulated in the wild-type strain in response to light, whereas only 26 were down-regulated, considering a threshold of threefold change. Genes strongly induced by light included genes known to be up-regulated by light, like the carotenogenic gene *carB* (74-fold), cryptochrome (45-fold) and *mcwc-1c* (22-fold), supporting reliability of the microarray data. Although many of up-regulated genes code for proteins implicated in protection against light-induced damage, several of them code for protein involved in signal transduction that could be involved in light responses like phototropism. Transcriptomic analysis of *mcwc-1* mutants showed that induction of around 60% of the genes is mediated by *mcwc-1a*, whereas only 1% is mediated by *mcwc-1c* and none is mediated by *mcwc-1b*, suggesting that *mcwc-1a* is the main photoreceptor. Searching for cis-acting regulatory motifs upstream of genes regulated by *mcwc-1a* identified consensus sequences similar to those found in light regulated genes of *Neurospora crassa*. Moreover, the identification of a small group of genes regulated by the three *mcwc-1* genes points out that the three proteins form complexes to regulate gene expression. Funded by MINECO (BFU2012-32246), Spain.

437. Gene expression profiling of the basidiomycetous fungus *Lentinula edodes* after light stimulation. H. Sano[1], Y. Sakamoto[2], M. Abe[3], S. Kaneko[4], M. Nakamura[1], Y. Miyazaki[1]. 1) Department of Applied Microbiology, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan; 2) Department of Biological Resources Research, Iwate Biological Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003,Japan; 3) Forest and Forestry Research Institute, Tokushima Agriculture, Forestry and Fisheries Technology Support Center, 5-69 Nanshocho, Tokushima, Tokushima 770-0045, Japan; 4) Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsutacho, Midori-ku, Yokohama, Kanagawa 226-8503, Japan.

Light is an important environmental signal for various organisms and is known to regulate their physiological and developmental processes. In fungi, light influences sexual and asexual reproductions, mycotoxin productions and pigmentation. Morphological differentiations of mushroom-forming homobasidiomycetes are also affected by light and stimulation of light is one of the important factors regulating fruiting body formation. The molecular mechanisms regulated by light are well studied in ascomycetes, and a number of light-regulated genes are identified and are characterized. In basidiomycetes, the photoreceptor-encoding genes homologous to *Neurospora crassa wc-1* have been isolated from several species and those genetical evidences revealed the involvement in fruiting body formation. However, the details of light-sensing systems and light-dependent regulation of genes are unclear. In this study, we performed super serial analysis of gene expression (SuperSAGE) using Illumina/Solexa genetic analyzer and analyzed the change of gene expressions stimulated by light during fruiting body formation in *Lentinula edodes*. The samples for high-throughput SuperSAGE were prepared from mycelia cultivated under darkness and after exposure to light with low temperature treatment. The mycelium which had been exposed to light formed a number of primordium and developed into normal fruiting bodies, whereas the mycelium cultivated under darkness could produce no primordium. The obtained transcriptome data showed that there were many kinds of genes expressed in *L. edodes* after light irradiation (5251 genes), compared with the data under darkness (2876 genes). The comparison analysis revealed that the expressions of 2500 genes were different between light and dark condition, and that over 2000 genes were strongly transcribed in *L. edodes* after exposure to light. Light irradiation also caused the decrease in expression levels of 500 genes. The up- and down-regulated genes were categorized by Gene Ontology and were assigned by the KEGG pathway mapping. These analyses suggested that several genes encoding putative fungal-specific proteins were regulated by light. The cataloged data of the expressed genes provide valuable information on understanding of light-sensing system in *L. edodes*.

438. Further Characterization of Surface Recognition Mechanisms in Magnaporthe oryzae. Guanghui Wang[1], Xiaoying Zhou[2], Guotian Li[2], Jin-Rong Xu[1,2]. 1) College of Plant protection, Northwest A & F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue Universtiy, West Lafayette, USA.

Surface recognition and appressorium penetration are critical infection processes in the rice blast fungus Magnaporthe oryzae and many other plant pathogenic fungi. Various chemical and physical surface signals are known to be recognized by germ tubes to activate the Pmk1 MAP kinase that is conserved in fungal pathogens for regulating appressorium formation and penetration. Recently, the Msb2 mucin gene was found to function as a surface sensor upstream from the Pmk1 pathway. However, it is not clear how is Msb2 activated and what is its relationship with other surface sensors. In this study, we found that the cleavage domain and transmembrane helics are essential for Msb2 functions. Site-directly mutagenesis was used to verify two candidate cleavage amino acid sites. In addition, we conducted deletion analysis with the cytoplasmic tail of Msb2 that likely plays a role in intracellular signaling. We also assayed the effects of over-expressing the C-terminal region of Msb2 and identified proteins co-precipitated with it by affinity purification. Because CBP1 and PTH11 are two other putative surface sensor genes, we also generated the msb2 cbp1 and msb2 pth11 double mutants and triple mutants with sho1. The msb2 cbp1 mutant rarely formed appressoria and was non-pathogenic, indicating that Msb2 and Cbp1, the only two mucins in M. oryzae, may have overlapping functions in surface recognition. Detailed phenotype characterization of the msb2 pth11 and triple mutants are under the way. A model of Msb2 activation and relationship among different receptors will be presented.

439. Evidence of Microbial Epigenetics; Loss-of-function mutant of the Bck1 Homolog, CpBCK1, from the chestnut blight fungus *Cryphonectria parasitica* resulted in the sectoring accompanied with the changes in DNA methylation. J.-M. Kim[1], S.-H. Yun[2], K.-Y. Jahng[2], D.-H. Kim[2]. 1) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea; 2) Institute for Molecular Biology and Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea.

*Cpbck*1, encoding a mitogen-activated protein (MAP) kinase kinase kinase from the chestnut blight fungus *Cryphonectria parasitica*, is an ortholog of

Bck1 from *Saccharomyces cerevisiae*. Colony morphology of the *Cpbck*1-null mutants differed dramatically from the wild type that mutants showed the invasive growth pattern characterized by slower growth rate, absence of distinctive aerial hyphae resulting in almost absence of conidia-bearing structure and conidia, sparse mycelial growth on the surface of agar plate with abnormal pigmentation, and irregular mycelial mat within the restricted area. Feeding hyphae growing under the plate showed less branched and relatively slower growth pattern. Interestingly, the *Cpbck*1-null mutant produced sectors appeared as thick rubbery patches of matted growth without pigmentation and sporulation. Complementation of the *Cpbck*1-null mutant with a wild-type allele rescued mutant phenotypes indicating that the mutant phenotypes were due to the absence of the *Cpbck*1 gene. Intracellular structure observed by electron microscope revealed both invasive growth-type and sectored-type showed the occurrence of hypertrophy of cell wall, multiple nuclei within swollen cells and intrahyphal hyphae. DNA methylation, an indicative of epigenetic marker, examined by Southern blot analysis and bisulfite DNA modification of putative target genes revealed that there was difference in the DNA methylation pattern between original *Cpbck*1-null mutant and sectored isolate. This study suggests that epigenetic changes are predisposed by the loss of function mutation of a specific gene *Cpbck*1 and it will be of interest to determine what decide the transition of the mycelia growth pattern from the invasive and very-sick hyphal growth type to compact-mat type. The *Cpbck*1-null mutant showed the sectored phenotype accompanied with the changes in DNA methylation demonstrated that the fungal signaling pathway implicated in the control of epigenetic processes, without which abnormal degeneration such as sectoring occurred.

440. NUP-6 (Importin a) is required for DNA methylation in *Neurospora crassa*. Andrew D. Klocko[1], Michael R. Rountree[1], Paula L. Grisafi[1], Shan M. Hays[2], Eric U. Selker[1]. 1) Institute of Molecular Biology, University of Oregon, Eugene, OR 97448; 2) Department of Natural and Environmental Sciences, Western State College of Colorado, Gunnison, CO 81231.

Heterochromatic regions on chromosomes are essential for numerous cellular processes, including centromere function and gene silencing. The repetitive DNA found in heterochromatin is highly compacted, frequently A:T rich, and in some species such as *Neurospora crassa*, methylated at cytosines. This DNA methylation can effectively silence genes. Interestingly, unlike the situation in some eukaryotes, loss of DNA methylation is not required for viability in Neurospora. The dispensability of DNA methylation in Neurospora allows for the identification of **d**efective **i**n **m**ethylation (*dim*) genes that have critical roles in the establishment, maintenance, and/or regulation of DNA methylation. This approach established that, at least in Neurospora, DNA methylation is initiated by the histone methyltransferase activity of a multi-subunit complex, DCDC (DIM-5/-7/-9 CUL-4 DDB-1^{dim-8} Complex), which catalyzes tri-methylation of lysine 9 on histone H3 (H3K9me3). While the identification of the components of the DCDC was an important step to understanding heterochromatin formation, much is still unknown about the DCDC, including its detailed function, regulation, and assembly. Here, we characterize the action of a previously unidentified *dim* mutant, *dim-3*. We found that *dim-3* corresponds to the *nup-6* gene, which encodes the Importin a subunit (NUP-6) for classical nuclear transport. NUP-6^{dim-3} virtually abolishes H3K9me3 and significantly reduces DNA methylation, and causes DIM-5 and DIM-7 to be mislocalized from heterochromatin, suggesting DCDC activity is impacted in a *dim-3* strain. Curiously, nuclear transport of DCDC components in a *dim-3* strain background appears to be equal to or greater than in a wild type background. The possibility exists that NUP-6 could be important in directing the DCDC to heterochromatin or in assembly of the DCDC, and we will address these hypotheses. In addition, the mutations found in *dim-3* could prevent its gene product, NUP-6, from facilitating DCDC action.

441. Identification and characterization of a *Blastomyces dermatitidis* mutant with a bidirectional defect in the phase transition. Amber J. Marty, Gregory M. Gauthier. University of Wisconsin - Madison, 1550 Linden Drive, Microbial Sciences Building, Madison, WI, 53706.

Collectively, the dimorphic fungi are the most common cause of invasive fungal disease worldwide. The ability of these fungi to undergo a shift between mold and yeast is critical for pathogenesis. In the soil (22°C), these fungi grow as mold, which produce infectious conidia. Following soil disruption, aerosolized conidia inhaled into the lungs of a host (37°C) convert into yeast to cause pneumonia. Knowledge of the mechanisms used to regulate this phase transition is limited. To uncover genes that control the phase transition, *Agrobacterium tumefaciens*-mediated DNA transfer was used to mutagenize *Blastomyces dermatitidis* conidia. We generated and screened 22,000 insertional mutants for defects in the phase transition. We identified a mutant, 11-9-75, with a single site of T-DNA insertion that grew as pseudohyphae at 37°C and 22°C, rather than yeast or mold. Adaptor PCR, DNA sequencing, and reverse transcription PCR (RT-PCR) revealed the T-DNA was located in the 5' UTR of an uncharacterized gene (referred to as *BKY1*) that was not annotated in the *B. dermatitidis* genome. Analysis of cDNA indicated *BKY1* was 1546 bp in length, lacked introns, and the ORF was predicted to encode a 156 amino acid protein. BLAST analyses against the NCBI database failed to reveal homologs of *BKY1* in other fungi. The T-DNA insert altered transcription of *BKY1* in mutant 11-9-75. *BKY1* transcript in the mutant was 35-fold higher versus wild type (WT) by quantitative real-time PCR (qPCR) and truncated at the 5' UTR when analyzed by rapid amplification of cDNA ends (RACE). RT-PCR supported the qPCR and RACE analyses, and indicated the GAPDH promoter, which is upstream of a hygromycin resistance cassette in the T-DNA was driving increased transcription of truncated *BKY1*. The T-DNA insert also altered alternative splicing of a gene with unknown function, *Bd594*, which was less than 1.2 kb downstream of *BKY1*. Although transcript abundance of *Bd594* in mutant 11-9-75 was similar to WT, the frequency of intron excision was reduced. In conclusion, we have identified an insertional mutant with a bidirectional defect on the phase transition; it grows as pseudohyphae instead of yeast at 37°C or mold at 22°C. The T-DNA insert alters transcription of adjacent genes, *BKY1* and *Bd594*, in a poorly characterized region in the *B. dermatitidis* genome.

442. Transcriptional regulation of peptidases and nitrogen transporters during the assimilation of organic nitrogen by the ectomycorrhizal fungi *Paxillus involutus*. Firoz Shah[1], Francois Rineau[2], Tomas Johansson[1], Anders Tunlid[1]. 1) Microbial Ecology Group, Department of Biology, Lund University, SE-22362, Lund, Sweden; 2) Centre for Environmental Sciences, Hasselt University, Building D, Agoralaan, 3590 Diepenbeek, Limburg, Belgium.

Proteins and amino acids form a major part of the organic nitrogen (N) sources in soils. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the role of ectomycorrhizal extracellular peptidases and amino acid transporters in the degradation, uptake and transfer of various protein sources (BSA, Gliadin and pollen) as well as of plant litter material using the ECM model fungus *Paxillus involutus*. During N-deprived conditions, all substrates induced secretion of peptidase activities. The activity had acidic pH optimum (2.3-3.0), and it was mainly due to aspartic peptidases and with minor contribution of metallo and serine peptidases. The activity was partly and temporarily repressed by low concentrations of ammonium (1mg/L). Transcriptional analysis showed that *P. involutus* expressed a large array of proteins and enzymes involved in the assimilation of organic N including peptidases, N-transporters and enzymes of the N-metabolism. Extensive *in-silico* analysis revealed the presence of genes encoding 312 peptidases, 129 N transporters and 284 enzymes involved in amino acid metabolism. Out of these, 89 peptidases and 37 N-transporters and 109 amino acid metabolism enzymes encoding genes were significantly upregulated during organic N assimilation. The genes were encoding a variety of secreted (23) and non-secreted (20) peptidases which were differentially expressed depending on the medium with the highest expression of the aspartic and metallo peptidases. Apart from the YAAH/ATO family, upregulated genes were found in all the other families of transporters for amino acids, oligopeptides, ammonium, urea and allantoate/allantoin. The results shows that the expression levels of peptidases and transporters in *P. involutus* are coordinately regulated during the assimilation of organic N sources.

443. Characterization of genome maintenance components in Neurospora crassa using whole-genome high-throughput approach. Evelina Y. Basenko, Zack Lewis. Department of Microbiology, University of Georgia, Athens, GA.

Eukaryotic DNA is packaged into a higher order DNA-protein structure also known as chromatin, which can regulate and impact an array of nuclear processes including DNA repair and genome maintenance. Disruptions in genome integrity can lead to serious ailments in humans and also contribute to cancer emergence. DNA repair and genome maintenance have been extensively studied in yeast. We, however, have chosen to investigate genome maintenance in the filamentous fungus Neurospora crassa. N. crassa possesses a much larger genome than budding yeasts, and it also contains approximately 1400 genes which are conserved in higher eukaryotes including humans and are absent in yeasts. We utilized the whole-genome Neurospora knockout library to search for novel regulators of genome maintenance. We screened the knockout library for mutants sensitive to the DNA damage agent methyl methane sulfonate (MMS). We have further confirmed and investigated additional mutagen sensitivities of confirmed MMS-sensitive strains. For our further studies, we have decided to focus on one of the MMS-sensitive mutants, which contains a deletion of a SNF2-like protein. The whole-genome high-throughput approach is a powerful method to identify novel players of genome maintenance. We have identified several hundred mutants sensitive to DNA damage, that fall within various categories of cellular processes, including but not limited to DNA repair, RNA metabolism, and chromatin maintenance. Our findings and current progress will be reported.

444. Circadian regulation and carbon catabolite repression in Neurospora crassa: Two integrated regulatory systems? Rodrigo Díaz-Choque, Luis F Larrondo. Dept Molecular Genetics & Microbiology, Pontificia Universidad Católica de Chile, Santiago, Chile.

Circadian clocks are autonomous timers composed of interconnected transcriptional/transcriptional feedback loops. They are thought to confer a selective advantage to individuals by temporally coordinating several processes and contributing to cellular homeostasis. In Neurospora crassa, a model organism in circadian studies, ~20%; of its genes are under circadian control and interestingly; many of them are related to metabolism. Indeed, the idea of a crosstalk between metabolism and the circadian clock has become stronger in the last years, and several examples have been obtained in mammalian systems. However, we still don't know in Neurospora the actual influence of circadian regulation in its physiology and its real impact in the "real-world". Moreover, the different transcriptional regulators linking time-of-day information and the expression of genes involved in metabolically relevant processes, like Carbon-Catabolite Repression (CCR) or cellulose degradation remain largely unknown. Thus, we are analyzing glucose repression in a circadian context, using N. crassa as a model. We hypothesize that there is an intimate crosstalk between both regulatory systems over the expression of several rhythmic CCR-controlled genes. We are using gene expression assays and a codon-optimized luciferase transcriptional reporter, to evaluate the role of the transcription factor CRE-1 (carbon catabolite regulation-1), a conserved metabolic regulator, in this potential cross-talk. Also, CRE-1 is a crucial transcription factor involved in several important cellular processes as cellulose degradation and catabolic repression. Further, we are studying how both inputs are integrated to control the expression of target genes. Our results suggest CRE-1 as a link between both pathways, as it appears to be important for both CCR and circadian control of target genes. In addition, we describe for the first time In Neurospora the presence of a functional clock in cellulose (Avicel) -containing media. This observation strengthens the hypothesis that a circadian clock may regulate the expression of several cellulase-encoding genes, having a real impact in such a physiologically relevant process.

445. Opposing activities of the HCHC and DMM complexes maintain proper DNA methylation in *Neurospora crassa*. Shinji Honda[1,2], Eun Yu[1], Eric Selker[1]. 1) University of Oregon, Institute of Molecular Biology, Eugene, OR; 2) University of Fukui, Life Science Unite, Fukui, Japan.

Proper regulation of heterochromatin and DNA methylation is critical for the normal function of cells. We show that heterochromatin and DNA methylation are faithfully controlled in Neurospora by opposing activities of the silencing complex HCHC and the anti-silencing complex DMM. The workings of these two complexes were investigated. HCHC consists of four proteins, the two chromo domain proteins HP1 and CDP-2, the histone deacetylase HDA-1 and the AT-hook motif protein CHAP. We found that histone deacetylase activity is critical for HCHC function but the H3K9me3 binding activity of the CDP-2 chromo domain is not. Instead, CDP-2 serves as an essential bridge between HP1 and HDA-1. CHAP interacts directly with HDA-1, binds in a methylation-independent way to the A:T-rich DNA that forms the cores of methylated regions and is important for stable association of HDA-1 with chromatin. HCHC is involved in the spreading of DNA methylation in dmm mutants. The DMM complex consists of a presumed histone demethylase, DMM-1, plus DMM-2, which is characterized by a fungal-specific $Zn(II)_2Cys_6$ DNA-binding domain ("Zn-Cys"). We found that DMM-2 strongly binds to DNA from euchromatin/heterochromatin junctions, thereby promoting the stable association of DMM-1 at the edge of heterochromatin domains to prevent aberrant spreading of DNA methylation.

446. The transcription factor FL is phosphorylated and interacts with a trehalose related protein in *Neurospora crassa*. Carmen Ruger-Herreros[1], Gencer Sancar[2], Michael Brunner[2], Luis M. Corrochano[1]. 1) Departamento de Genetica, Universidad de Sevilla, Spain; 2) BZH, Universität Heidelberg, Germany.

Several environmental cues, including light, promote a developmental transition in *Neurospora crassa* that leads to the formation of conidia. Conidiation is controlled by FLUFFY (FL), a zinc finger transcription factor. Light activates the transcription of *fl* through the transient binding of the WC complex to the *fl* promoter. Light also activates the transcription of several conidiation genes in *Aspergillus nidulans*, and their *Neurospora* homologs have been identified

in the *Neurospora* genome. We have assayed the activation by light of the *Neurospora* homologs of *A. nidulans* conidiation genes (*flbA, flbC, flbD, medA* and *stuA*), and the *Neurospora* conidiation gene *con-10* as a control. Unlike *con-10*, none of the *Neurospora* homologs of the *A. nidulans* conidiation genes were induced by light in vegetative mycelia. However, we found that deletion *fl* resulted in light-dependent mRNA accumulation for all the conidiation genes. This result indicated that the absence of FL allows the binding of the WC complex to the promoter of these genes to activate transcription in a light-dependent manner. We have assayed the amount of WC proteins in the D*fl* and wild type strains but we did not find any difference between the two strains. We expect to identify additional genes deregulated by the absence of FL after massive sequencing of total RNA (RNAseq) using a D*fl* strain and wild-type strain in dark and light conditions. We have investigated the role of FL during conidiation in *Neurospora* using a tagged version of FL. FL is present in vegetative mycelia but the amount increses after light exposure. We observed several forms of FL due to phosphorylation, and and we have determined by mass spectrometry that FL is phosphorylated in several residues. We have immunoprecipitated FL to identify proteins that may interact with FL. We have found a protein that interacts with FL in different growth conditions. This protein has been described in other organisms and plays a role in the ability to grow in the presence of trehalose. Since FL is a transcription factor, we have use FL::3XFLAG strain to do ChIPseq in order to identify the putative binding sites of FL to the DNA. We expect that the results from these experiments will help us to understand in more detail the role of FL in the activation of gene transcription during development.

447. Transcriptomic profiling of fumonisin B biosynthesis by *Fusarium verticillioides*. N. Ponts, E. Zehraoui, L. Pinson-Gadais, F. Richard-Forget, C. Barreau. INRA, UR1264-MycSA, 71 avenue Edouard Bourlaux, BP81, F-33883 Villenave d'Ornon, France.

The plant fungal pathogen *Fusarium verticillioides* can infect various plants worldwide, including maize, and contaminate kernels with mycotoxins of the fumonisin family. Fumonisins B are stable polyketides that resist agrifood processing and are classified as potentially carcinogenic. As such, contamination of food and feeds with these toxic secondary metabolites must be avoided. Numerous factors influence fumonisins B accumulation on maize, including the composition of the grains on which *Fusarium* develops. In particular, several phenolic compounds were shown to inhibit fumonisin B biosynthesis. Preliminary analyses showed that free phenolic acids are particularly abundant in immature grains, *i.e.,* at the onset of toxin production, from cereal cultivars on which mycotoxins tend to accumulate less. We tested in vitro the effect of chlorogenic, caffeic, and ferulic acid on fumonisin B production in *F. verticillioides*. All three compounds inhibit fumonisin B accumulation, caffeic acid being the most efficient with that regard. We investigated the mechanisms by which these phenolic acids may exert their inhibitory properties and analyzed whole genome expression levels by RNA-seq. Sequenced reads were mapped to the reference genome of *F. verticillioides* and results were analyzed according to the current annotation available at the *Fusarium Comparative Database*. Doing so, we identified 175 and 1133 potential new genes and transcripts, respectively. We also found that the genes involved in the fumonisins biosynthetic pathway are all inhibited in the presence of any of the three tested phenolic acids. Finally, we identified sets of genes that are regulated specifically by a given phenolic acid, and others that follow similar patterns in all tested conditions. As a whole, our results show a large re-organization of *Fusarium*'s transcriptome upon phenolic acid treatment.

448. Differential transcriptome analysis of *Zymoseptoria tritici* infecting wheat reveals novel effectors. Stefano F.F. Torriani[1], Marcello Zala[1], Daniel Croll[1], Patrick C. Brunner[1], Eva H. Stukenbrock[2], Dee Carter[3], Bruce A. McDonald[1]. 1) Integrative Biology, ETHZ, Zurich, Switzerland; 2) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 3) University of Sydney, Sydney, Australia.

Zymoseptoria tritici (formerly called *Mycosphaerella graminicola*) is a hemibiotrophic fungus belonging to the Dothideomycetes, the largest class of ascomycetes that includes many plant pathogens. Like other hemibiotrophic pathogens *Z. tritici* uses different strategies for obtaining nutrition during its life cycle. For the first 10 days post inoculation (dpi) the pathogen colonize the host as a biotroph without causing visible symptoms. The necrotrophic phase lasts until the affected plant cells have died. Depending on the strain-cultivar interaction, plant cell death occurs from 18 to 20 days after penetration. *Z. tritici* concludes its life cycle by surviving as a saprotroph on dead leaves for several months. Thus *Z. tritici* presents a powerful system to study host-pathogen interactions during different stages of disease development. Next generation sequencing technology was used to analyze changes in transcription during the complete infection cycle of *Z. tritici* on wheat. The total RNA was extracted from inoculated plants at six time points (3-, 7-, 11-, 14-, 21- and 56- dpi). RNA-Seq analyses allowed us to trace the expression profile of 10,251 genes and identify genes that differed in expression between the biotrophic, necrotrophic and saprotrophic stages of infection. About 14% and 34% of the genes showed statistically significant differences in expression from the biotrophic to necrotrophic and from the necrotrophic to saprotrophic stages of infection, respectively. Putative effector genes were preferentially transcribed at 11 dpi during the transition between biotrophy and necrotrophy. Through this screen we identified five putative effector genes for further characterization, using Agrobacterium-mediated transformation to determine their role in pathogenicity. Although recent experimental efforts focused mainly on proteinaceous effectors, we investigated the role of non-proteinaceous metabolites as they can also manipulate host cells. Two different clusters of genes (PKS4 and PKS5-related genes) involved in the biosynthetic pathways of different secondary metabolites showed expression patterns similar to the putative effectors. Confirmation of function of the putative virulence genes will be based on gain or loss of virulence in planta using gene knock-outs and knock-ins.

449. Role of the *xprG* gene in autolysis, secondary metabolism and asexual development in *Aspergillus nidulans* . Margaret E. Katz, Katharyn Braunberger, Sarah Cooper. Dept Molec & Cellular Biol, Univ New England, Armidale, Australia.

The *Aspergillus nidulans xprG* gene encodes a transcriptional activator that is a member of the Ndt80 family in the p53-like superfamily of proteins. Previous studies have shown that XprG controls the production of extracellular proteases in response to starvation. We undertook transcriptional profiling to investigate whether XprG has a wider role as a global regulator of the carbon nutrient stress response. Our microarray data showed that the expression of a large number of genes, including genes involved in secondary metabolism, development, and autolysis, were altered in an *xprG*D null mutant. Many of these genes are known to be regulated in response to carbon starvation. We confirmed that sterigmatocystin and penicillin production is reduced in *xprG*- mutants. The loss of fungal mass and secretion of pigments that accompanies fungal autolysis in response to nutrient depletion was accelerated in an *xprG1* gain-of-function mutant and decreased or absent in an *xprG*- mutant. We found that conidophore development occurred in carbon-starved submerged cultures of both the *xprGD1* loss- and *xprG1* gain-of-function mutants, though the number of metulae appeared to be reduced. Thus, the reduction of *brlA* expression observed in the *xprGD1* mutant is not sufficient to block conidiophore development in response to carbon starvation. . However, the *xprG1* gain-of-function mutation partially suppresses VeA-mediated repression of conidiophore development and the conidiophore development defect in the fluG701 mutant. These results support the hypothesis that XprG plays a major role in the response to carbon limitation and that nutrient sensing may represent one of the ancestral roles for the p53-like superfamily.

450. Fugal-specific sirtuin *HstD* coordinates the secondary metabolism and development via the *LaeA*. M. Kawauchi[1,2], K. Iwashita[1,2]. 1) Dept. Mol. Biotech., Grad. Sch. Adv. Sci. Mat., Hiroshima Univ., Hiroshima, Japan; 2) Natl. Res. Inst. Brewing, Hiroshima, Japan.

The sirtuins are members of the NAD^+-dependent histone deacetylase family that contribute to various cellular functions which are affected aging, disease and cancer development. However, physiological roles of the fungal-specific sirtuin family are still poorly understood, especially with regard to their participation in the genomic stability of yeast. Here, we determined the novel function of the fungal-specific sirtuin *HstD*, which is homolog of yeast *Hst4* in *Aspergillus oryzae*. The deletion of *HstD* indicated that both conidial development and secondary metabolism were regulated by *HstD* in *A. oryzae*. Furthermore, the gene expression of *LaeA*, which is the most studied coordinator for the regulation of secondary metabolism and development, was induced in the *DHstD* strain, and we found a significant genetic interaction between *HstD* and *LaeA* using double-disrupted or overexpression strains. Thus, we concluded the fungal-specific sirtuin *HstD* coordinates the fungal development and secondary metabolism via the regulation of *LaeA* gene expression in filamentous fungi. The *HstD* is fungal-specific, but it is conserved in the vast family of filamentous fungi. Therefore, *HstD* has great potential as a drug target for mycosis or plant disease, because the fungal development and secondary metabolism are virulence determinants of pathogenic fungi. In addition, our findings are also important for improving the productivity of useful secondary metabolites and developing an attractive host for the production of several heterogeneous secondary metabolites.

451. Improved flavor production by manipulation of the Ehrlich pathway in ascomycetes. D. Ravasio, A. Walther, J. Wendland. Carlsberg Laboratory, Copenhagen V, Denmark.

The Ehrlich pathway utilizes amino acids to generate higher alcohols with distinctive flavor in three enzymatic steps including a transaminase, a decarboxylase and an aldehyde dehydrogenase. Comparative genomics revealed the absence of key genes of the Ehrlich pathway in Eremothecium cymbalariae whereas these genes were found to be present in the closely related species Ashbya gossypii. A. gossypii produces a very fruity flavor both in liquid culture and on solid media. The biological significance of this is unknown. Here, we present the functional analysis of A. gossypii key genes of the Ehrlich pathway, ARO8a, ARO8b, ARO10, and ARO80. Deletion of any one component resulted in a noticeable reduction of flavor production as determined by GC/MS. In Saccharomyces cerevisiae ARO80 has been described as the main transcription factor regulating other genes of the Ehrlich pathway. Therefore, we analyzed the effect of deletion and overexpression of this gene on flavor production in yeast. As expected, overexpression resulted in a marked increase in flavor production, particularly in isoamyl alcohol, a banana-like flavor. Next to chemical analyses we generated a lacZ-based reporter gene assay using ARO-gene promoters. With such a tool we can determine the status of flavor production under various conditions and in a variety of yeast strains. Initial results will be presented.

452. Suppressor mutagenesis of a *DlaeA* mutant reveals novel regulators of secondary metabolism in *Aspergillus nidulans*. Alexandra Soukup, Jerry Luo, Jin Woo Bok, Nancy P. Keller. UW-Madison, Madison, WI.

Aspergillus nidulans is a filamentous fungus known to produce a variety of complex natural products known as secondary metabolites (SM). Regulation of these bioactive SM can occur through cluster specific transcription factors, or through global regulators such as LaeA. Deletion of *laeA* results in drastically decreased amounts of multiple secondary metabolites. A multi-copy suppressor screen for genes capable of phenotypically returning norsolorinic acid (NOR) production to the *DlaeA* mutant resulted in identification of 17 plasmids containing inserts ranging from one to four genes. Further analysis of the suppressor plasmids confirmed of a subset to increase SM production both in the original *laeA* deletion strain and in wild type backgrounds.

453. A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*. J. Aït-Benkhali[1,2], E. Coppin[1,2], S. Brun[1,2,3], T. Martin[4], C. Dixelius[4], R. Debuchy[1,2]. 1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) UFR des Sciences du Vivant, Université Paris-7 Diderot, Paris, France; 4) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden.

High-mobility group B proteins are eukaryotic DNA-binding proteins characterized by the HMG-box functional motif. These transcription factors play a pivotal role in global genomic functions and in the control of genes involved in specific developmental or metabolic pathways. The filamentous ascomycete *Podospora anserina* contains 12 HMG-box genes. Of these, four have been previously characterized; three are mating-type genes that control fertilization and development of the fruiting-body, whereas the last one encodes a factor involved in mitochondrial DNA stability. Systematic deletion analysis of the eight remaining uncharacterized HMG-box genes indicated that none were essential for viability, but that seven were involved in the sexual cycle. Two HMG-box transcription factors display striking features. Pa_1_13940, an ortholog of SpSte11 from *Schizosaccharomyces pombe*, is a pivotal activator of mating-type genes in *P. anserina*, whereas Pa_7_7190 is a repressor of several phenomena specific to the stationary phase, most notably hyphal anastomoses. Constitutive expression of mating-type genes in a *DPa_1_13940* strain did not restore fertility, indicating that *Pa_1_13940* has additional functions related to sexual reproduction besides activating mating-type genes. RT-qPCR analyses of HMG-box genes in different HMG-box deletion strains indicated that *Pa_1_13940* is at the hub of a network of several HMG-box factors that regulate the sexual cycle. Complementation experiments with a strain deleted for mating-type genes revealed that this network control fertility genes in addition to mating-type target genes. This study points to the critical role of the HMG-box members in sexual reproduction in fungi, as 11 out of 12 members were involved in the sexual cycle in *P. anserina*. Pa_1_13940 and SpSte11 are conserved transcriptional regulators of mating-type genes, although *P. anserina* and *S. pombe* have diverged 1.1 billion years ago. Two HMG-box genes, *SOX9* and its upstream regulator *SRY*, play also an important role in sex determination in mammals. The mating-type genes and their upstream regulatory factor form a module of HMG-box genes similar to the *SRY/SOX9* module, suggesting it may be ancestral in Opisthokonta.

454. *Sclerotinia sclerotiorum* MAT genes function in fertility and apothecial morphogenesis. Benjamin Doughan, Jeffrey Rollins. Plant Pathology, University of Florida, Gainesville, FL.

Sclerotinia sclerotiorum (Lib.) de Bary is an omnivorous, polyphagus, phytopathogenic fungus that relies on the completion of the sexual cycle to initiate most new disease cycles. The sexual cycle is characterized by the development of apothecia that forcibly discharge ascospores for local and, under suitable conditions, long distance dissemination. A strategy for understanding the regulation of apothecial multicellular development is being pursued through functional characterization of the mating type genes in *S. sclerotiorum*. These genes are hypothesized to encode master regulatory proteins required for aspects of sexual development ranging from fertilization through fertile fruiting body development. Experimentally, gene deletion strategies were performed to create loss-of-function mutants in the two conserved “core” mating type genes common to most ascomycete fungi as well for two lineage-specific genes found only in *S. sclerotiorum* and closely related fungi. *mat 1-1-1* and *mat 1-2-1* mutants are able to form ascogonia but are blocked in all aspects of apothecia development. These mutants also exhibit defects in secondary sexual characters including the production of smaller sclerotia and lower numbers of spermatia. *mat 1-2-4* mutants are delayed in apothecia production and form apothecia with aberrant disc morphogenesis and ascospore production. They too produce lower numbers of spermatia and smaller sclerotia and additionally, exhibit a slower hyphal growth rate. Phenotypes of the *mat 1-1-5* gene knockouts are under evaluation and will be reported. Our findings demonstrate that mat genes are involved in both sexual fertility and development in *S. sclerotiorum*.

455. The *Sclerotinia sclerotiorum* mating type locus (*MAT*) contains a 3.6-kb region that is inverted in every generation. Patrik Inderbitzin[1], Periasamy Chitrampalam[2], Karunakaran Maruthachalam[1], Bo-Ming Wu[3], Krishna Subbarao[1]. 1) Department of Plant Pathology, University of California-Davis, Davis, CA, USA; 2) Department of Plant Sciences, University of Arizona, Tucson, AZ, USA; 3) Department of Plant Pathology, China Agricultural University, 2 West Yuanmingyuan Rd., Haidian District, Beijing, China.

Sclerotinia sclerotiorum is a filamentous ascomycete in the *Sclerotiniaceae* (Pezizomycotina) and a necrotrophic pathogen of more than 400 hosts worldwide, including many important agricultural crops. In California, the biggest lettuce producer in the United States, *S. sclerotiorum* is a causal agent of lettuce drop that reduces overall annual lettuce yield by 15%. Little is known about the details of sexual reproduction in *S. sclerotiorum*, but the structure of the *S. sclerotiorum* mating type locus *MAT*, the master regulator of sexual reproduction in ascomycetes, has previously been reported. As in other homothallic (self-fertile) ascomycetes, *S. sclerotiorum MAT* contains both idiomorphs (divergent alleles) fused end-to-end at a single locus. Using 283 isolates from lettuce in California and from other states and hosts, we investigated the diversity of *S. sclerotiorum MAT*, and identified a novel version of *MAT* that differed by a 3.6-kb inversion and was designated Inv+, as opposed to the previously known *S. sclerotiorum MAT* that lacked the inversion and was Inv-. The inversion affected three of the four *MAT* genes: *MAT1-2-1* and *MAT1-2-4* were inverted and *MAT1-1-1* was truncated at the 3'-end. Expression of *MAT* genes differed between Inv+ and Inv- isolates. In Inv+ isolates, only one of the three *MAT1-2-1* transcript variants of Inv- isolates was detected, and the alpha1 domain of Inv+ *MAT1-1-1* transcripts was truncated. Both Inv- and Inv+ isolates were self-fertile, and the inversion segregated in a 1:1 ratio regardless of whether the parent was Inv- or Inv+. This suggested the involvement of a highly regulated process in maintaining equal proportions of Inv- and Inv+, likely associated with the sexual state. The MAT inversion region, defined as the 3.6-kb *MAT* inversion in Inv+ isolates and the homologous region of Inv- isolates, was flanked by a 250-bp inverted repeat on either side. The 250-bp inverted repeat was a partial *MAT1-1-1* that through mediation of loop formation and crossing over, may be involved in the inversion process. Inv+ isolates were widespread, and in California and Nebraska constituted half of the isolates examined. We speculate that a similar inversion region may be involved in mating type switching in the filamentous ascomycetes *Chromocrea spinulosa*, *Sclerotinia trifoliorum* and in certain *Ceratocystis* species.

456. Repression of the phosphor-transmitter gene *ypdA* resulting in growth defect in *Aspergillus fumigatus*. Daisuke Hagiwara[1], Hiroki Takahashi[1], Mayumi Nakayama[2], Keietsu Abe[2], Tohru Gonoi[1], Susumu Kawamoto[1]. 1) Medical Mycology Research Center, Chiba university, Chiba, Japan; 2) NICHe, Tohoku university, Sendai, Japan.

Two-component system (TCS) is a conserved signal transduction system implicated in cellular responses to a variety of environmental stimuli in fungi. *Aspergillus fumigatus* has 13 histidine kinases, single HPt (histidine-containing phosphor transmitter), and 3 response regulators, which together constitute a TCS signaling. According to studies of HPt in several fungi, *ypdA* encoding a single HPt of *A. fumigatus* has been thought to be an essential gene. In this study, we tested if absence of YpdA leads to cell lethality in *A. fumigatus*, and investigated what the molecular mechanisms underlying the lethality is. To address these questions, we constructed a conditional *ypdA*-expressing strain by replacing a native promoter of *ypdA* with *thiA* promoter (P*thiA*). P*thiA* is strictly repressed in the presence of thiamine, while moderately expressed in the absence of thiamine. The conditional P*thiA*-*ypdA* strain showed severe growth defect on a plate containing thiamine, while the strain grew normally on a plate without thiamine as the wild-type stain. We, then, investigated the expression profiles of *catA*, *dprA*, and *dprB* genes, which are regulated under the control of SakA MAPK cascade, since the SakA MAPK cascade is downstream of *A. fumigatus* TCS. In a liquid culture, expressions of *catA*, *dprA*, and *dprB* were gradually increased after addition of thiamine, suggesting that inactivation of *ypdA* expression leads to the activation of SakA MAPK cascade. To get more insight into a response to *ypdA*-repression, the transcriptome profiles were obtained by RNA-seq. Expression levels of each gene at 3h, 6h, and 9h after addition of thiamine were compared to that at 0h. More than 2-fold or less than 1/2-fold of expression changes were regarded as up- or down-regulated, respectively. Through statistical analysis on category of gene ontology, the groups concerning ribosome biogenesis or RNA metabolism were found to be significantly down-regulated after the inactivation of *ypdA*. Based on these results, we will discuss the cellular responses to YpdA deprivation and try to find out the molecular mechanisms attributed to the lethality.

457. Unravelling the GTPase polarity complex in *Claviceps purpurea*. Andrea Herrmann[1], Janine Schürmann[1], Britta Tillmann[2], Michael Bölker[2], Paul Tudzynski[1]. 1) IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany; 2) Philipps-Universität, Karl-von-Frisch-Strasse 8, 35032 Marburg, Germany.

Claviceps purpurea is a plant pathogen infamous for its production of toxic alkaloids on infected host plants like barley. Consumption of infected grains leads to severe symptoms up to the death of the patient. Infection patterns are complex and the topic of intensive research. One interesting aspect is the strict polarity of the hyphal growth during the first infection stage which seems to be crucial for the non-recognition of *C. purpurea* as a pathogen by the host. To address the question of the importance of polarity the structure and dynamics of the polarity complex are the focus of this work. The guanine nucleotide exchange factors (GEFs) Cdc24 and Dock180 belong to different families, Cdc24 being a member of the Dbl GEF family and Dock180 a CZH GEF. Cdc24-GFP localises cytosolically and to hyphal tips whereas Dock180-GFP is present in small vesicles in the hypha, though concentrated at the tip region, too. Cdc24 DHPH domains are able to activate the small GTPases Rac and Cdc42 of *C. purpurea* and *U. maydis In vitro*, whereas the catalytic domain of Dock180 only activates Rac in both organisms. Despite the proven activation Cdc24 does not interact with any GTPase in yeast two hybrid assays. Dock180 shows a weak interaction with Rac and the two p21-activated kinases (PAKs) Ste20 and Cla4. Thus, both GEFs do not share many characteristics apart from their GEF activity. The PAKs Ste20 and Cla4 and the scaffold protein Bem1 are involved in the polarity complex, too. Ste20 localises to hyphal tips and interacts with Cdc42 in a loading status dependent manner, whereas Cla4 is the main partner of Rac. Other interactions of Ste20 with Dock180 and Cla4 could also be shown. Bem1 is present in the cytosol - concentrated at the hyphal tip - and links most of the proteins of the polarity complex as interactions with Cdc24, Cla4, Ste20 and Dock180 have been detected. Taken together we postulate at least two different polarity complexes, the Rac complex and the Cdc42 complex. Both are gathered by Bem1, but Cla4 is the main partner of Rac, whereas Ste20 plays a similar role for Cdc42. Dock180 is mainly linked to Rac, Cdc24 can be active in both complexes. We are interested in the spatial and temporal formation and regulation of these complexes and its influence on polarity and virulence which will be the subject of further studies.

458. Atypical Gb and RACK homolog Gib2 is a signal transducing adaptor protein affecting growth and virulence of Cryptococcus neoformans. Yanli Wang[1], Gui Shen[1], Jinjun Gong[1], Amy Whittington[2], Ping Wang[1,2,3]. 1) Res Inst for Children, Children's Hospital, New Orleans, LA USA; 2) Dept Microbiology, Immunology and Parasitology, LSUHSC, New Orleans, LA USA; 3) Dept Pediatrics, LSUHSC, New Orleans, LA USA.

Virulence in Cryptococcus neoformans is a multifaceted trait underpinned by complex signaling pathways. The atypical G-protein b subunit Gib2 displays versatility in interactions with signaling molecules such as Ga Gpa1 that governs cAMP signaling and intersectin Cin1 that regulates intracellular trafficking. This and the conserved seven-bladed b-propeller motif are highly suggestive that Gib2 functions as an adaptor protein. We here show that Gib2 binds to

Gpa1 and Gpg1/Gpg2 in a direct manner, and Gib2 promotes cAMP levels through novel interactions with phosphodiesterase Pde2 and RAS proteins Ras1 and Ras2. Using TAP technology, we have identified additional 42 proteins whose putative function range from signal transduction, energy generation, metabolism, and stress response to ribosomal function. Finally, through establishing a protein-protein interactive network, we illustrate that Gib2 adapts a scaffold role to mediate specific protein-protein interactions that drive the formation of various protein complexes. This includes fostering a heterotrimeric complex with Gpa1 and Gpg1/Gpg2, targeting Pde2 (direct) and adenylyl cyclase Cac1 (indirect) to regulate cAMP levels, and likely serving as a conserved ribosomal core protein facilitating fundamental cellular processes to underlie growth and virulence. Our studies reveal the complexity of the regulatory network in fungi and advocate Gib2 as a novel target for antifungal therapy.

459. Consequences of the loss of transcription factors SreA and HapX on siderophore biosynthesis and iron homeostasis in the perennial ryegrass endophyte, *Epichloë festucae*. Natasha T. Forester[1,2], Geoffrey A. Lane[1], Iain L. Lamont[2], Linda J. Johnson[1]. 1) Plant Fungal Interactions Team, AgResearch Ltd, Palmerston North, New Zealand; 2) Biochemistry Dept, University of Otago, Dunedin, NZ.

Siderophores are low molecular weight ferric iron chelators that are made by microorganisms to compete for and to sequester iron, an essential micronutrient. *Epichloë festucae*, a fungal endosymbiont of perennial ryegrass, synthesises two siderophores, epichloënin A and ferricrocin to harvest and utilise iron from its host grass. Work by our group has implicated epichloënin A in the maintenance of symbiosis and is described in another abstract. To explore the regulation of siderophore biosynthesis and iron homeostasis processes, we have characterised mutants of two major iron-responsive transcription factors, SreA and HapX that coordinate cellular responses to iron availability. To evaluate the effect of loss of SreA and HapX on siderophore biosynthesis, we measured the production of epichloënin A and ferricrocin by LC-MS/MS. Relative to wild type; both siderophores were over-produced in D*sreA* mycelia grown in the presence of iron, while ferricrocin was produced in excess in D*hapX* mycelia grown under iron deprived conditions. Iron-dependent phenotypic deviations from wild type fungal growth were also observed in culture and in planta but neither mutant disrupted the *E. festucae* - *L. perenne* association under standard soil conditions. However, under iron-limiting conditions through hydroponic control of symbiotic iron supply, we demonstrated that D*sreA* mutants can induce chlorosis in their hosts, indicating that D*sreA* mutants compete for host iron. In planta, the D*sreA* fungal hyphae are also markedly increased in girth and lack growth in vascular bundles. In D*hapX* infected plants grown hydroponically in iron deprived conditions, we observed inappropriate fungal growth such as highly convoluted and compressed hyphae and elongated fungal structures which hinted at reduced resource conservation by D*hapX* under growth limiting conditions. Collectively, these results suggest that Epichloë fungi have a tightly regulated iron management system for niche adaptation and actively set limits on iron withdrawal from the host, presumably to prevent competition with its host to promote mutualistic interactions. Mutations that interfere with fungal iron management, either by deregulating siderophore synthesis, can destabilise the fungal-plant association.

460. Who is to blame: defining the host responses that lead to ToxA-induced susceptibility. Iovanna Pandelova, Viola Manning, Ashley Chu, Lynda Ciuffetti. Botany and Plant Pathology, Oregon State Univ, Corvallis, OR.

Pathogenicity by the necrotrophic pathogen of wheat, *Pyrenophora tritici-repentis (Ptr)*, is attributed to the production of host-selective toxins (HSTs). Understanding the mode-of-action of HSTs is essential for a complete characterization of how these pathogenicity factors condition plant disease susceptibility. One of the proteinaceous HSTs produced by *Ptr*, PtrToxA (ToxA), induces necrosis in sensitive cultivars. Several studies suggest that ToxA interacts with a high affinity receptor, enters mesophyll cells and localizes to chloroplasts. Additionally, ToxA acts as an elicitor of defense responses by increasing production of phenolic compounds and by the up-regulation of genes involved in jasmonic acid and ethylene production pathways. After ToxA treatment and incubation in constant light, there is a decrease in photosystem (PS) I and II transcripts observable already at 9 and 14 hours post infiltration (hpi), which is followed by the drastic reduction in levels of both PSI- and PSII-complex proteins. It is proposed that photosystem dysfunction leads to light-dependent accumulation of reactive oxygen species (ROS) and the development of necrosis. To better understand the role of ROS, photosynthesis and defense responses in necrosis development induced by ToxA, plants were incubated in light (presence of ROS) or in dark (absence of ROS). In order to determine the impact of ToxA on gene regulation and to establish when early changes in protein content of PS complexes occur, both biochemical and microarray analyses were performed. Some defense-related genes are up-regulated in ToxA-treated leaves incubated in the dark (ToxA/dark), although the number of probesets was considerably less compared to ToxA-treated leaves incubated in light (ToxA/light). Furthermore, ethylene biosynthesis genes, that play a role in symptom development in ToxA/light treatrment are not significantly up-regulated in ToxA/dark-treated leaves. Finally, only a small fraction of PSI- and II-related and chlorophyll a/b-binding genes are down-regulated in ToxA/dark compared to ToxA/light treatment. These data suggest that only certain defense-related pathways are involved in ToxA-induced necrosis development, and help to identify those genes whose differential regulation by ToxA is light and/or ROS-dependent.

461. RNA silencing of *pacC* increases *aflR* transcript levels under alkaline pH conditions in *Aspergillus flavus*. Benesh M Somai, Kyle W van der Holst, Essa Suleman. Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, 6031, Eastern Cape, South Africa.

Aspergillus flavus produces aflatoxin B1 which is an important hepatocarcinogen, especially amongst the developing third world countries which have a large number of poor, rural, subsistence communities with little access to fungicides. The master regulator of aflatoxin production is *aflR* which, in turn, appears to be negatively regulated by *pacC*. However, until now, there were never any direct measurements of the relative *aflR/pacC* transcript ratios produced under aflatoxin conducive and non-conducive conditions. In the current study, *pacC* was down-regulated in two transformants by a synthetic *pacCRNAi* construct under the control of a thiamine inducible promoter. Expression of *pacC* and *aflR* transcripts was then measured via RT-qPCR in cultures grown under alkaline or acid conditions. At pH 4, between *pacCRNAi* inducing and repressing conditions, an *aflR/pacC* transcript ratio of 1.09 relative to the reference gene was obtained indicating the production of an equal abundance of *aflR* and *pacC* mRNA. It is generally accepted that at acidic pH the majority of *pacC* mRNA is unprocessed, remains untranslated and non-functional thereby being incapable of repressing AFLR protein production. This stimulates aflatoxin production at acidic pH. Between pH 8 and pH 4, when *pacCRNAi* was suppressed, the *aflR/pacC* ratio was 0.2 indicating that *pacC* production was higher than that of *aflR*. *aflR* transcript levels were reduced between 76% and 80% therefore explaining the normal lack of aflatoxin detection at pH 8. Between pH 8 and pH 4, when *pacCRNAi* was induced, the *aflR/pacC* ratio was between 1.77 and 13.21 indicating that at alkaline pH, suppression of *pacC* allowed a large increase in AFLR which stimulated aflatoxin production. It is concluded that *pacC* is produced at acidic pH, but remains largely non-functional. Furthermore, at pH 8, *aflR* production decreases only by about 80% and therefore it is possible that the remaining 20% of transcripts still stimulates aflatoxin production. Finally, via RNAi silencing it is conclusively proved that *pacC* negatively regulates *aflR* production at pH 8.

462. High-throughput prediction and functional validation of promoter motifs regulating gene expression in spore and infection stages of *Phytophthora infestans*. H. Judelson, S. Roy, M Kagda. Dept of Plant Pathology and Microbiology, University of California, Riverside, CA.

Most filamentous pathogens have complex life cycles in which gene expression networks orchestrate the formation of cells specialized for dissemination

or host colonization. In the oomycete *Phytophthora infestans,* the potato late blight pathogen, we identified major shifts in mRNA profiles during developmental transitions using microarrays. We then used those data with three search algorithms to discover more than 100 motifs that are over-represented in promoters of genes up-regulated in hyphae, sporangia, sporangia undergoing zoosporogenesis, swimming zoospores, or germinated cysts forming appressoria. Most of the putative stage-specific transcription factor binding sites (TFBSs) thus identified had features typical of TFBSs such as position or orientation bias, palindromy, and conservation in related species. Each of six motifs tested in *P. infestans* transformants using the GUS reporter gene conferred the expected stage-specific expression pattern, and were shown to bind nuclear proteins in gel-shift assays. Several motifs linked to the appressoria-forming stage were over-represented in promoters of genes encoding effectors and other pathogenesis-related proteins. To understand how promoter and genome architecture influence expression, we also mapped transcription patterns to the *P. infestans* genome assembly. Adjacent genes were not typically induced in the same stage, including genes transcribed from a small shared promoter region. Analyses of global expression, however, demonstrated that co-regulated gene pairs occurred more than expected by random chance. These data help illuminate the processes regulating development and pathogenesis, and will enable future attempts to purify the cognate transcription factors. Our approach should be applicable to both oomycetes and fungi.

463. Cooperative regulation of *Aspergillus nidulans* cellulase genes by transcription factors McmA and ManR/ClrB. Tetsuo Kobayashi[1], Nuo Li[1], Miki Aoyama[1], Yohei Yamakawa[1], Masahiro Ogawa[2], Yasuji Koyama[2]. 1) Grad Sch of Bioagricultural Sci, Nagoya Univ, Nagoya, Aichi, Japan; 2) Kikkoman Corp, Noda, Chiba, Japan.

Expression of the endoglucanase A gene (*eglA*) in *A. nidulans* is inducible by cellobiose. Previously, the *cis*-element responsible for the inductive expression, designated CeRE (Cellulose Responsive Element), was identified based on mutational analysis of the *eglA* promoter. CeRE contained the binding consensus of SRF-MADS proteins, suggesting involvement of McmA, the sole SRF-MADS protein in *A. nidulans*. While two Zn_2Cys_6 transcription factors in *Aspergillus* were recently reported to be essential to cellulase induction. One is ManR in *A. oryzae*, which regulates both mannanase and cellulase genes, and the other is ClrB in *A. nidulans*, a homolog of the cellulase regulator CLR-2 in *N. crassa*. Since these factors were orthologous sharing 63% identity, we use the name ManR/ClrB. In this presentation, we provide evidences that McmA and ManR/ClrB cooperatively regulate induction of cellulase genes. Effects of *mcmA* mutation and *manR*/*clrB* deletion on expression of cellulase genes were examined by qRT-PCR. Expression of *eglA*, *eglB*, and *cbhA* was highly induced by cellobiose in the wild type strain. The induction was significantly impaired by the *mcmA* mutation and abolished by the *manR*/*clrB* deletion, indicating that the cellulase genes under control of McmA and ManR/ClrB are overlapped. Binding of His-tagged McmA and Flag-tagged ManR/ClrB-DBD (DNA Binding Domain), which were produced in *E. coli* and purified, to the CeRE containing region of the *eglA* promoter was examined by EMSA. McmA gave two shifted bands corresponding to single and double occupation of the binding sites, which lay within and just upstream of CeRE. ManR/ClrB-DBD alone showed very weak binding to the region. When both McmA and ManR/ClrB-DBD were added, a slower-migrating and more abundant shifted band was appeared, which suggested cooperative binding of McmA and ManR/ClrB-DBD. Presence of McmA and ManR/ClrB-DBD in the shifted band was confirmed by supershift assay with the anti-his-tag and anti-flag-tag antibodies. These results indicated that inductive expression of *eglA* is regulated by cooperative binding of McmA and ManR/ClrB to its promoter, and suggests that regulation of *eglB* and *cbhA* would be similar. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

464. Transcription factor shuttling during cellulase induction in *Trichoderma reesei*. Alex Lichius, Christian P. Kubicek, Verena Seidl-Seiboth. Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria.

For economically feasible production of liquid fuels and other value-added compounds from lignocellulosic plant material, strategies are required to boost cellulolytic and hemicellulolytic enzyme production by industrially relevant fungi. One promising approach is to modulate the transcriptional control mediating release from carbon catabolite repression (CCR) and induction of cellulase, hemicellulase and xylanase gene expression. To better understand the underlying molecular dynamics during induction, we characterized nucleo-cytoplamic shuttling of the two transcription factors carbon catabolite repressor 1 (CRE1) and xylanase regulator 1 (XYR1) of *Trichoderma reesei* by means of live-cell imaging. In submerged cultures, nuclear import and export of CRE1 upon repression and induction, respectively, occurred within minutes and therefore was generally faster than shuttling of XYR1. Under CCR conditions XYR1 expression levels were very low, and its nuclear signal required up to one hour to significantly increase upon replacement into an inducing carbon source. Cultured directly under inducing conditions, nuclear accumulation of XYR1 was detectable after about 20h post inoculation, and strongly increased within the following 24 hours. CRE1 under the same conditions was localized exclusively to the cytoplasm. In plate cultures, nuclear recruitment of CRE1 and XYR1 differed within the central area, the subperiphery and the periphery of the colony depending on the provided carbon source. Most interestingly, under inducing conditions we found evidence for increased nuclear recruitment of CRE1 in the central area, correlating with strong nuclear import of XYR1 in the same region. Notably, the cytoplasmic signal of CRE1 was usually elevated in leading hyphae, whereas XYR1 was never significantly recruited to the colony periphery. Taken together our data provide the first temporal resolution of transcription factor shuttling during the induction of cellulase gene expression in *Trichoderma reesei*, and reveal some interesting differences between the subcellular localization of CRE1 and XYR1 in submerged and plate cultures, respectively. These differences indicate that the mycelial organization during fungal growth might be another important regulatory element to consider for the industrial scale production of cellulolytic enzymes.

465. *Trichophyton rubrum ap-1* gene expression in response to environmental challenges. Nalu TA Peres, Gabriela F Persinoti, Larissa G Silva, Tiago R Jacob, Antonio Rossi, Nilce M Martinez-Rossi. School of Medicine, Univiversity of Sao Paulo, Ribeirao Preto, Brazil.

Several families of transcription factors (TF) are found in fungal cells, which contribute for the broad range of cellular responses triggered by environmental changes in these organisms. These TF regulate the expression of genes involved in different cellular processes allowing cell survival under stressful as well as physiological conditions. The AP-1 TF belongs to the bZIP family (basic leucine zipper), and is involved in the conidiation process, response to oxidative stress, multidrug resistance, and pathogenicity of some fungi. In dermatophytes, a group of keratinophilic fungi, little is known about this TF and the processes in which it is required. *Trichophyton rubrum* is the major etiologic agent isolated from clinical cases of cutaneous mycoses in humans, and studies of the responses of this fungus to several environmental conditions allow a better understanding of its physiology and pathogenicity, thus providing information of how to decrease its growth and to establish more efficient therapeutic measures. Here, we evaluated the *ap-1* gene expression profile during growth of *T. rubrum* in several nutrient sources (keratin, *ex vivo* skin and nail), exposure to antifungal drugs, and oxidative/osmotic stresses. An up-regulation of *ap-1* gene expression was observed during *ex vivo* infection and keratin utilization, compared to growth in a glucose containing medium. In response to different antifungal agents, *ap-1* was up-regulated, while osmotic and oxidative stress did not alter its expression level. These results provide insights into the regulation of the *ap-1* TF gene expression, suggesting its involvement in *T. rubrum* pathogenicity, possibly regulating several genes that allow the utilization of proteins from the host tissues as nutrient sources, and also protecting the cell against the damages caused by antifungal drugs. Financial Support: FAPESP, CNPq, CAPES, and FAEPA.

466. Vib-1 is required for cellulose utilization in Neurospora crassa. Yi Xiong, Jianping Sun, N. Louise Glass. Department of Plant and Microbial Biology, Univ California, Berkeley, Berkeley, CA.

Vib-1 (vegetative incompatibility blocked) encodes a transcriptional regulator that is required for nonself recognition and heterokaryon incompatibility in Neurospora crassa. It is also required for the production of extracellular proteases upon carbon and nitrogen starvation. We found that vib-1 null mutant was severely defective in utilizing cellulose as a carbon source and this growth defect could be rescued by constitutively expressing clr-2, an essential transcription factor for cellulase gene expression. Our transcriptional profiling with RNA-seq showed that most genes that were induced in wild type on cellulose were not induced in the vib-1 null mutant, but were expressed in vib-1 null mutant under non-inducing conditions when clr-2 transcription factor was constitutively expressed. Our data suggests that vib-1 functions in a signaling pathway upstream of clr-2 for cellulose utilization. By comparing the transcriptomes of vib-1 and clr1/clr-2 mutants versus wild type in response to different carbon sources including sucrose and cellulosic materials as well as carbon and nitrogen starvations, we differentiated vib-1 dependent gene expression into several categories. We propose that vib-1 may play an important role in carbon starvation signaling ,thus positively regulating preparation for utilization of cellulosic materials.

467. The stringency of start codon selection in the filamentous fungus *Neurospora crassa*. Jiajie Wei[1], Ying Zhang[1], Ivaylo P. Ivanov[2], Matthew S. Sachs[1]. 1) Dept Biol, Texas A&M Univ, College Station, TX; 2) BioSciences Institute, University College Cork, Ireland.

In eukaryotic cells, initiation may occur from near-cognate codons that differ from AUG by a single nucleotide. The stringency of start codon selection impacts the efficiency of initiation at near-cognate codons and the efficiency of initiation at AUG codons in different contexts. We used a codon-optimized firefly luciferase reporter initiated with AUG or each of the nine near-cognate codons in preferred context to examine the stringency of start codon selection in the model filamentous fungus *Neurospora crassa*. *In vivo* results indicated that the hierarchy of initiation at start codons in N. crassa (AUG >> CUG > GUG > ACG > AUA » UUG > AUU > AUC) is similar to that in human cells. Similar results were obtained by translating mRNAs in a homologous *N. crassa in vitro* translation system or in rabbit reticulocyte lysate. We next examined the efficiency of initiation at AUG, CUG and UUG codons in different contexts *in vitro*. The preferred context was more important for efficient initiation from near-cognate codons than from AUG. These studies demonstrated that near-cognate codons are used for initiation in *N. crassa*. Such events could provide additional coding capacity or have regulatory functions. Analyses of the 5'-leader regions in the *N. crassa* transcriptome revealed examples of highly conserved near-cognate codons in preferred contexts that could extend the N-termini of the predicted polypeptides.

468. A temperature-dependent complex transcriptional network controls cell shape and virulence in *Histoplasma capsulatum*. Sinem Beyhan[1], Matias Gutierrez[1], Mark Voorhies[1], Anita Sil[1,2]. 1) Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Histoplasma capsulatum, which is a respiratory fungal pathogen of humans, is endemic in the United States. Depending on the exposure dose and the immune status of the host, the infection can lead to mild-respiratory or life-threatening and systemic disease. *H. capsulatum* has a dimorphic life cycle, switching from an infectious filamentous form in the soil to a pathogenic yeast form in mammalian hosts. This morphological switch, which requires a dramatic shift in the gene expression profile of the cells, can be easily recapitulated in the laboratory simply by changing the temperature from room temperature to 37°C. We previously identified three regulators, Ryp1, Ryp2 and Ryp3, which are required for the yeast-phase growth. *ryp1*, *ryp2* and *ryp3* mutants are unable to respond to change in temperature and grow constitutively in the filamentous form even at 37°C. Ryp1 belongs to a conserved family of fungal proteins that regulate cellular differentiation in response to environmental signals. The best-studied member of this family of proteins is Wor1, which is a master regulator of white-to-opaque switching in *Candida albicans*. Ryp2 and Ryp3 are orthologous to VosA and VelB, respectively, which are developmental regulators in *Aspergillus nidulans*. In this study, using transcriptional profiling and chromatin immunoprecipitation (ChIP) experiments, we explored complementary and unique roles of Ryp1, Ryp2, and Ryp3 in regulating yeast-phase growth. Our results reveal that Ryp1, Ryp2 and Ryp3 physically interact and associate with DNA throughout the genome. Additionally, we identified a fourth transcription factor, Ryp4, which is a direct target of Ryp1, Ryp2 and Ryp3, as a novel regulator of yeast-phase growth in *H. capsulatum*. Further transcriptional profiling and ChIP experiments show that Ryp4 regulates and associates with the upstream regions of a subset of Ryp1, Ryp2, and Ryp3 targets, which are involved in morphology and virulence in *H. capsulatum*. Finally, we identified two distinct *cis*-regulatory elements that are utilized by Ryp1 or the Ryp2/Ryp3 complex to facilitate gene expression. Our results reveal a tightly regulated and interwoven transcriptional network that controls the ability of a pathogenic fungus to cause disease in response to host temperature.

469. Evolutionary analysis of Dicer proteins: a preliminary analysis to study of microRNAs in the mushroom, Coprinopsis cinerea. Xuanjin Cheng, Hoi Shan Kwan. Life Sciences, The Chinese University of Hong Kong, New Territory, Hong Kong.

Coprinopsis cinerea is a mushroom of limited edible value and is extensively used as a model organism to study the development of homobasidiomycete fungi. Unraveling the molecular basis of the fungus developmental processes would contribute to evolutionary studies and lead to improvement in the breeding and cultivation of edible or medical homobasidiomycete mushrooms. MicroRNA (miRNA) is a group of endogenous non-coding regulatory RNAs of ~22 nt that regulate gene expression in various biological processes such as cell differentiation, development regulation and heterochromatin formation. Dicer is a key enzyme involved in the biogenesis of miRNAs and is highly conserved through eukaryotes. A miRNA-like RNA cannot be defined as a miRNA unless a Dicer (or Dicer-like) protein is found participating in its biogenesis. There are three Dicer homologs (CC1G_00230, CC1G_03181, CC1G_13988) identified in the C. cinerea genome. In order to gain an insight into the roles of Dicer proteins in C. cinerea and to investigate whether Dicer is involved in miRNA biogenesis, we employed a comprehensive phylogenetic analysis of the Dicer protein family in all of the three kingdoms under Eukaryota - animal, plant and fungus - and highlighted the results of Dicer homologs in C. cinerea. We showed that Dicer genes duplicated and diversified independently in early animal, plant and fungus evolution, coincident with the origins of multicellularity. Besides, identified a group of Dicer homologs that are specific to mushroom-forming fungi. We also showed that changes in one of the Dicer domains, the double-stranded RNA binding domain (dsRBD), alone may lead to diversification of Dicer proteins. As a whole, we revealed a dynamic picture in which the evolution of Dicer proteins has driven elaboration of parallel RNAi functional pathways in the animal, plant and fungus kingdoms.

470. Effect of the *trp1* gene on transformation frequencies in *Coprinopsis cinerea*. Bastian Doernte, Ursula Kües. Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Germany.

Genetic transformation of the basidiomycete *Coprinopsis cinerea* has first been described by Binninger et al. in 1987 (1). For the transfer of genetic material, chromosomal integrative vectors are used, which contain a selectable marker gene and/or a gene of interest. During transformation the genetic material integrates at ectopic sites into the host chromosomes. Binninger et al. (1987) created the vector pCc1001, by cloning a 6.5 kb *Pst*I genomic

fragment, with the tryptophan synthetase gene (*trp1*) of *C. cinerea*, into the ColE1 vector pUC9. The inserted gene allows the complementation of *trp1* auxotrophies and can be used as a selection marker. Several transformation experiments using this vector reveal a surprising phenomenon. Single-transformation with solely pCc1001 gives only low numbers of transformants, whereas co-transformation with an additional plasmid yields about 2x more transformants. To explore this phenomenon, the length of the *trp1* harboring fragment was changed and the existing replicon was replaced by a modified ColE1 replicon. All single-transformations resulted in the same observations. An alternative selection marker (*pab1* encoding) and different relative vector-vector concentrations were tested in several co-transformation experiments. The obtained results lead to the conclusion that tryptophan feedback inhibition might be responsible for the reduced transformation efficiencies in single-transformations of *trp1* vectors. (1) Binninger et al. (1987). DNA-mediated transformation of the basidiomycete *Coprinus cinereus*. EMBO J 6:835-840.

471. The first promoter for conditional gene expression in *Acremonium chrysogenum*: iron starvation-inducible *mir1^P*. Fabio Gsaller[1], Michael Blatzer[1], Beate Abt[1], Markus Schrettl[2], Herbert Lindner[3], Hubertus Haas[1]. 1) Christian Doppler Laboratory for Fungal Biotechnology, Division of Molecular Biology, Medical University of Innsbruck, Austria; 2) Sandoz GmbH, Kundl, Austria; 3) Division of Clinical Biochemistry, Medical University of Innsbruck, Austria.

The filamentous fungus *Acremonium chrysogenum* is of enormous biotechnological importance as it represents the natural producer of the beta-lactam antibiotic cephalosporin C. However, a limitation in genetic tools, e.g. promoters for conditional gene expression, impedes genetic engineering of this fungus. Here we demonstrate that in *A. chrysogenum* iron starvation induces the production of the extracellular siderophores dimerumic acid, coprogen B, 2-N-methylcoprogen B and dimethylcoprogen as well as expression of the putative siderophore transporter gene, *mir1*. Moreover, we show that the promoter of *mir1*, *mir1^P*, is suitable for conditional expression of target genes in *A. chrysogenum* as shown by *mir1^P*-driven and iron starvation-induced expression of genes encoding green fluorescence protein and phleomycin resistance. The obtained iron-starvation dependent phleomycin resistance indicates the potential use of this promoter for selection marker recycling. Together with easy scorable siderophore production, the co-regulation of *mir1* expression and siderophore production facilitates the optimization of the inducing conditions of this expression system. This work was funded by Sandoz GmbH (Kundl, Austria) and the Christian Doppler Society (Vienna, Austria).

472. Mutagenic effect of high-LET ion beam irradiation in *Neurospora crassa*. Ligiu Ma[1*], Yusuke Kazama[2], Tomoko Abe[2], Shuuitsu Tanaka[1], Shin Hatakeyama[1]. 1) Regulation Biol, Saitama Univ, SAITAMA, Japan; 2) Radiation Biology Team, RIKEN, SAITAMA, Japan.

Heavy ion beams cause great damages to cellular components particularly generating severe DNA damages, DNA double strand breaks (DSBs). We examined the biological effect and mutagenesis of irradiation of high-LET ion beam (Fe-ion) to DSB repair defect mutants in filamentous fungus *Neuospora crassa*. Fe-ion beam ($^{56}Fe^{24+}$: 90 MeV/u, LET=641 keV/mm) was irradiated to two DSB repair deficient mutants and wild-type strain. By lower doses (<100 Gy), all of the strains showed same survival rates. In the case of higher doses (>100 Gy), sensitivity to irradiation of the *mus-52* strain (non-homologous end-joining deficient) is higher than that of the wild type, whilst lower than that of the *mei-3* strain (homologous recombination deficient). Frequency of forward mutation occurred in the *ad-3* loci was similar to previously examined C-ion beam irradiation, i.e. *mei-3* > wild type > *mus-52* strains. However, characteristic difference of mutation was observed as the scale of deletions; large deletions were frequently in the Fe-ion beam irradiated wild type strain, comparing to that 1 bp-deletions were mainly observed in the C-ion irradiation. Differences of mutagenesis and killing effect between the irradiation of two heavy ions, Fe-ion and C-ion, were discussed based on types of DNA damages.

473. The Mad complex binds to light-regulated promoters in Phycomyces blakesleeanus. Alejandro Miralles-Duran, LM Corrochano. Genetica, Facultad de Biologia, University of Sevilla, Sevilla, Spain.

The zygomycete Phycomyces blakesleeanus responses to light include phototropism of the fruiting body, activation of beta-carotene biosynthesis, and regulation of fruit body development. These photoresponses require the Mad complex, a protein complex composed of proteins MadA and MadB. These proteins are homologous of WC-1 and WC-2 from Neurospora crassa and presumably play a similar role in the regulation by light of gene expression. MadA and MadB have a zinc finger domain at the carboxyl end, and MadA has a LOV domain that should serve as the binding site for a flavin chromophore. In Phycomyces, the Mad complex should operate as a photoreceptor and transcription factor complex. The Phycomyces genome contains two additional wc-1 homologs, wcoA and wcoB, and three additional wc-2 homologs, wctB, wctC, and wctD, but their function is unknown. We have expressed MadA and MadB in E. coli, and we have shown that these proteins bind the promoter of the light-regulated gene hspA by electrophoresis mobility shift assays (EMSA). Protein binding to the hspA promoter was observed with each isolated protein or with the two proteins associated in the Mad complex. The binding site to the hspA promoter will be identified by DNA footprinting analysis. We are performing similar assays with the other Phycomyces Wc proteins and we hope that the results will help us to understand the role of the multiple Wc proteins in light-dependent gene regulation in Phycomyces.

474. Down Regulation of sidB Gene by Use of RNA interference Technology in the Filamentous Fungi Aspergillus nidulans. S, Rezaie[1,2], H, Eslami[1], M.R. Khorramizadeh[1], M.R. Pourmand[1], M. Moazeni[2]. 1) Medical Biotechnology Dept, Tehran University of Medical Sciences, PhD; 2) Div. of Molecular Biology, Dept. of Medical Mycology and Parasitology, Tehran University of Medical Sciences, PhD.

Background: RNA interference (RNAi) is a natural process by which short double-stranded RNA (siRNA) silences the expression of complementary target RNAs by inducing RNA cleavage and subsequent reduction in protein expression levels. Introduction of the RNA interference machinery has guided the researchers to discover novel methodologies for knocking down essential vital factor or virulence factor genes in the microorganisms such as fungi. In filamentous fungi, Aspergillus nidulans, the gene sidB plays essential role in septation, conidiation and vegetative hyphal growth. In the present study, we benefited from the RNA interference strategy for down-regulating of a vital gene in the fungus Aspergillus nidulans. Materials and Methods: The 21-nucleotide siRNA was designed on the basis of the cDNA sequence of the sidB gene of A. nidulans. Transfection was performed via uptaking siRNAs from medium by germinated spores. After 18 hours of incubation, total RNA was extracted and quantitative changes in expression of the sidB gene were analyzed by measuring the cognate sidB mRNA level by use of a quantitative real-time RT-PCR assay. Results: In the presence of 25 nM of siRNA, a significant inhibition in germ tube elongation was observed compared with positive control samples (21 VS 42 mM). In addition, at the concentration of 25 nM , a considerable decrease in sidB gene expression was revealed. Conclusion: Usage of RNA interference as a kind of post-transcriptional gene silencing methods is a promising approach for designing new antifungal agents and discovering new drug delivery systems.

475. SmallRNA mediated meiotic silencing of a transposable element in Neurospora crassa. Yizhou Wang, Jason E. Stajich. Plant Pathology & Microbiology, Univ. of CA, Riverside, Riverside, CA.

Meiotic silencing of unpaired DNA plays an important role in protecting the genome integrity of Neurospora crassa. It is thought to fight against the invasion of virus and endogenous transposable elements. Our previous work has shown that a 10 KB MULE (mutator-like element)-related DNA

transposable element, named sly-1, uniquely exists in the wild type strain OR74A (FGSC#2489) of N. crassa. Here we show that in the cross between OR74A and D60 (FGSC#8820), a strain lacking sly-1, the unpaired sly-1 induced the production of small RNAs 4 days after fertilization. The small RNAs were generated from both strands of the sly-1 region and demonstrated typical Dicer-processed smallRNA features in Neurospora crassa: 25bp long with a strong preference for uridine at the 5' end. An RNA-dependent RNA polymerase (SAD-1) was found to be required for such small RNA production (1). We generated draft genome sequencing of D60 with Illumina HiSeq and compared it to the OR74A genome to identify additional unique regions where meiotic silencing of unpaired DNA may have occurred. These unique regions were also found to produce smallRNA with the same features as those from sly-1. These results provide strong support for the endogenous silencing role of meiotic silencing against a natural intact transposable element and describe the RNA interference pathway-involved silencing pattern of meiotic silencing. 1) Shiu PK, Raju NB, Zickler D, Metzenberg RL. Cell 2001; 107(7):905-16.

Pathogenic and Mutalistic Interactions

476. Functional Characterization of Small, Cysteine-Rich Secreted Effectors from the Filamentous Fungus Magnaporthe oryzae. William C. Sharpee, Yeonyee Oh, Bill Franck, Ralph A. Dean. Plant Pathology, NC State University, Raleigh, NC.

The filamentous fungus Magnaporthe oryzae is the most destructive pathogen of rice worldwide. It is described as having two distinct lifestyles within the host plant: a biotrophic phase during the early stages of infection followed by a necrotrophic phase characterized by host cell death and lesion formation. To identify candidate effector proteins that may contribute to pathogenesis, the genome of M. oryzae strain 70-15 was mined for predicted proteins that contain a signal peptide, have greater than 3% cysteine content, and are less than 250 amino acids in length. These criteria were selected based upon the characteristics of known effectors from other plant-pathogenic fungi and oomycetes. To investigate the roles of these candidates in the biotrophic or necrotrophic phases of infection, they were transiently expressed in Nicotiana benthamiana leaves via agroinfiltration. When expressed within plant cells, candidate effectors that induce necrosis in the N. benthamiana leaves could potentially act as inducers of host cell death during the necrotrophic phase of infection. Conversely, candidate effectors that prevent necrosis when co-infiltrated with known inducers of host cell death are potentially involved in suppressing host plant defenses and therefore may contribute to the biotrophic phase of infection. Of 70 candidate effectors tested to date, 10 were found to induce necrosis when transiently expressed in N. benthamiana. In addition, to test for suppression of host cell death, candidate effectors are currently being co-agroinfiltrated with the BAX gene, a known inducer of host cell death in both plant and mammalian cells, or a known necrosis inducer from M. oryzae. Those candidates that show an interesting phenotype will be selected for further characterization as potential effectors by analyzing their expression in planta and activity when expressed within rice protoplasts.

477. Penetration-specific effectors from *Phytophthora parasitica* favour plant infection. Edouard Evangelisti[1*], Benjamin Govetto[2], Naima Minet-Kebdani[1], Marie-Line Kuhn[1], Agnes Attard[1], Franck Panabieres[1], Mathieu Gourgues[1]. 1) UMR Institut Sophia Agrobiotech, INRA/CNRS/Université de Nice, Sophia Antipolis, France; 2) Institut Méditerranéen de Biodiversité et d'Écologie marine et continentale (IMBE), CNRS-INEE - IRD -Aix Marseille Université - Université d'Avignon - Institut Pytheas.

Oomycetes are major crop pests which cause million dollars losses every year. To date only a few efficient chemicals are available against these filamentous microorganisms. A better understanding of the molecular events occuring during plant-oomycete interactions will help to propose new strategies for crop protection. We performed a transcriptional analysis in order to identify oomycete penetration-specific genes and identified a set of penetration-specific effectors (PSE) bearing a RXLR motif. This motif was previously shown to promote effector import into plant cells during the biotrophic stage in feeding structures called haustoria. Here we report the functional analysis of three candidate genes, referred to as PSE1, PSE2 and PSE3. The three effectors were able to abolish plant defense responses when transiently expressed in Nicotiana plants. Moreover, constitutive expression of PSE1 and PSE3 in *A. thaliana* led to an enhanced susceptibility to *P. parasitica* infection suggesting a role for these proteins in *P. parasitica* pathogenicity. Transgenic Arabidopsis lines accumulating PSE1 protein showed several developmental perturbations that were associated with altered auxin physiology. Root growth inhibition assays showed that auxin signaling pathway is not altered by PSE1 accumulation. Nevertheless, the coiled-root phenotype and the enhanced susceptibility of PSE1-expressing lines to *P. parasitica* were reverted by synthetic auxin 2,4-D supply, or treatment with the auxin efflux inhibitor TIBA suggesting that a reduced auxin accumulation is responsible for these phenotypes. This hypothesis was confirmed by a reduced activity of the pDR5 auxin sensitive promoter at the root apex. The alteration of the expression pattern observed for two auxin efflux carriers, PIN4 and PIN7 suggests that a perturbation of auxin efflux could be responsible for the PSE1 associated defects. We proposed that PSE1 could favour *P. parasitica* virulence by interfering with auxin content. Our results show that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.

478. Transcriptional regulatory circuits necessary for appressorium-mediated plant infection by *Magnaporthe oryzae*. Miriam Oses- Ruiz, Darren M. Soanes, Nicholas J. Talbot. University of Exeter, Exeter, United Kingdom.

Rice blast disease is caused by the fungus Magnaporthe oryzae and is the most destructive disease of cultivated rice. The pathogen elaborates a specialized infection structure called the appressorium. The morphological and physiological transitions that lead to appressorium formation of M. oryzae during plant infection are stimulated through perception of environmental signals including surface hydrophobicity and hardness, and the presence of cutin monomers and leaf surface waxes. The fungus perceives and internalizes these stimuli by a variety of intracellular MAP kinase signaling pathways. The homeobox and C2/H2 Zn finger domain transcription factor, MST12 (ScSte12 homogue) is part of the PMK1 MAP kinase signalling pathway, which is required for appressorium formation and invasion. The Mst12 null mutant is able to form completely normal melanised appressoria but it is non pathogenic. The Mst12 null mutant is unable to form a penetration peg and therefore to cause disease in the rice plant. To understand the mechanism of the penetration peg formation, we have recently carried out genome-wide comparative transcriptional profiling analysis for mst12 null mutant using RNA-seq and HiSeq 2000 sequencing. In this way, we will show the transcriptional signature associated with penetration peg differentiation in the rice blast fungus. Moreover we will show the set of genes that are likely to be MST12 regulated and therefore help define the regulatory circuits necessary for appressorium-mediated plant infection by plant pathogenic fungi.

479. Differential activation of ammonium transporters during the accumulation of ammonia by *Colletotrichum gloeosporioides* and its effect on appressoria formation and pathogenicity. Dov B. Prusky[1], Chen Shnaiderman[1], Itay Miyara[1], Ilana Kobiler[1], Sherman Amir[2]. 1) Post Harvest Sci, Agricultural Res Org, Bet Dagan, Israel; 2) Genomic Unit, Plant Sciences Institute, ARO, Bet Dagan, Israel.

Ammonium secreted by the post-harvest pathogen *Colletotrichum gloeosporioides* during host colonization accumulates in the host environment due to

enhanced fungal nitrogen metabolism. Two types of ammonium transporter encoding genes, AMET and MEP, are expressed during pathogenicity. Gene disruption of AMET- a gene modulating ammonia secretion, showed twofold reduced ammonia secretion and 45% less colonization on avocado fruits, suggesting a contribution to pathogenicity. MEPB a gene modulating ammonium transport is expressed by *C. gloeosporioides* during pathogenicity and starvation conditions in culture. Gene disruption of MEPB, the most highly expressed gene of the MEP family, resulted in twofold overexpression of MEPA and MEPC but reduced colonization, suggesting MEPB expression's contribution to pathogenicity. Analysis of internal and external ammonia accumulation by DmepB strains in mycelia and germinated spores showed rapid uptake and accumulation, and reduced secretion of ammonia in the mutant vs. WT strains. Ammonia uptake by the WT germinating spores, but not by the DmepB strain with compromised ammonium transport, activated cAMP and transcription of PKA subunits PKAR and PKA2. DmepB mutants showed 75% less appressorium formation and colonization than the WT, which was partially restored by 10 mM exogenous ammonia. Thus while both AMET and MEPB genes modulate ammonia secretion, only MEPB contribute to ammonia accumulation by mycelia and germinating spores that activates the cAMP pathways, inducing the morphogenetic processes contributing to *C. gloeosporioides* pathogenicity.

480. Functional analysis of Nbs1 of Magnaporthe oryzae. K. Sasaki[1], K. Amano[1], T. Sone[2], M. Narukawa[1], T. Kamakura[1]. 1) Applied Biological Science, Tokyo Univ. of Science, Noda, Chiba, Japan; 2) Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

The filamentous fungus Magnaporthe oryzae causes rice blast, the most serious disease that affects global rice production. On the surface of host plant, a specialized infection structure called appressorium is formed on tip of germ tube. Induction of the development of appressorium requires several external stimulants and a complete cycle of cell division. Although many studies have revealed some of process of appressorium formation in M. oryzae, the complete mechanism is still obscure. We selected Nbs1 from germ tube expressing cDNA library and made Nbs1 disruptants. The cDNA library mainly contains the genes that express in the period of germ tube development and/or appressorium formation. Nbs1 is presumed to have forkhead associated (FHA) domain, which is contained in many proteins that are involved in DNA repair and cell cycle. In our previous study, Nbs1 disruptants showed growth delay, abnormality of conidia formation and nuclear division, reduction of germination rate and appressorium formation rate, abnormal pigmentation and high sensitivity to DNA-damaging agents. Although Neurospora crassa knock-out mutants of rcaA, which share sequence similarities with Nbs1, showed similar phenotypes to Nbs1 disruptants, rcaA did not seem to contain FHA domain. Toward further study of the function of Nbs1, we induced a plasmid carrying an rcaA (pNB51) or FHA domain-deleted Nbs1 (pCB51dF) into Nbs1 disruptants. Consequently, pNB51 and pCB51dF were able to partially complement phenotypes of Nbs1 disruptants. This result suggested that rcaA has at least partial similar functions of Nbs1 in N. crassa and another functional domain exists in Nbs1.

481. Influence of hypoxia on antifungal susceptibility, sterole pattern and biomarker release of Aspergillus spp. Ulrike Binder[1], Elisabeth Maurer[1], Christoph Müller[2], Franz Bracher[2], Cornelia Lass-Flörl[1]. 1) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Tirol, Austria; 2) Department of Pharmacy, Ludwig Maximilians University Munich, Germany.

Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates from 40% up to 90% in high-risk populations. The most common species causing aspergillosis is Aspergillus (A.) fumigatus, accounting for approximately 90% of infections. Depending on regional distinctions, A. flavus and A. terreus are frequently reported. During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO2 levels. Such oxystress conditions are usually not taken into account in current in vitro models of infection, the assessment of antifungal sensitivities or the release of biomarkers used for diagnosis. Therefore, we compared the in vitro activity of amphotericin B (amB), different azoles and echinocandins in hypoxic conditions (1% O2, 5% CO2) to their activity in normoxic conditions against isolates of A. fumigatus and A. terreus and other aspergilli. Using Etest strips, we found a reduction of the minimal inhibitory concentration (MIC) for amB for all aspergilli in hypoxic conditions. Similarly, a significant reduction in the MIC for all tested azoles was demonstrated for A. terreus isolates, while for A. fumigatus isolates differences were less pronounced. For echinocandins, little or no change in the MEC (minimal effective concentration) was detected between hypoxic and normoxic conditions for all aspergilli. Most interestingly, A. terreus strains, that are resistant to amB in normoxia, exhibited sensitivity to amB in hypoxic conditions, defining a breakpoint of > 2 mg/ml. Notably, for none of the strains tested, MIC/MEC values increased in hypoxia. Currently we are investigating if changes in the sterole pattern or the amount of ergosterol contribute to these changes in antifungal susceptibility in hypoxia. The detection of circulating fungal antigens in serum for Aspergillus galactomannan or b-D-glucan has become an accepted diagnostic strategy. However, sensitivity and specificity vary extremely and the reasons are only partially clear; therefore, we are currently checking whether hypoxia influences the physiological kinetics of GM and b-glucan release.

482. Sit and wait: Special features of *Aspergillus terreus* in macrophage interactions and virulence. M. Brock[1], I.D. Jacobsen[2]. 1) Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knoell Institute, Jena, Germany; 2) Molecular Pathogenicity Mechanisms, Hans Knoell Institute Jena, Germany.

While *Aspergillus fumigatus* is known as the main cause of invasive pulmonary aspergillosis in immunocompromised patients, *Aspergillus terreus* is an emerging pathogen prevalent in some local hot spots. When tested in embryonated egg or murine infection models *A. terreus* required substantially higher infectious doses compared to *A. fumigatus* to cause high mortality rates. Furthermore, when *A. fumigatus* and *A. terreus* infections were followed by *in vivo* imaging using bioluminescent reporter strains, germination and tissue invasion of *A. terreus* was significantly delayed. To elucidate differences in more detail, the interaction of *A. terreus* and *A. fumigatus* with macrophages was compared. *A. terreus* was phagocytosed significantly faster, which appears mainly due to higher exposure of galactomannan and glucans on the surface of conidia. Additionally, although phagocytosis of both species resulted in phagolysosome maturation, *A. fumigatus* efficiently inhibited acidification, which was not the case for *A. terreus*. However, within this acidic environment of phagolysosomes *A. terreus* showed long-term persistence without significant inactivation of conidia. Further analyses revealed that inefficient blocking of acidification by *A. terreus* was due to differences in the spore colour pigment of both species. Recombinant production of a naphthopyrone synthase from *Aspergillus nidulans* enabled *A. terreus* to inhibit the acidification to a similar extent as observed for *A. fumigatus*. This alteration of the phagolysosomal environment resulted in an increased escape from macrophages and was accompanied by increased virulence in a murine infection model. We speculate that the long-term persistence of *A. terreus* wild-type strains in acidified phagolysosomes might be responsible for high dissemination rates observed in infected human patients, because *A. terreus* might hitchhike inside immune effector cells to reach secondary sites of infection.

483. Identification and characterization of an RXLR-like effector family from medically relevant fungi. Shiv D. Kale[1*], Kelly C. Drews[1,2], Helen R. Clark[1,3], Hua Wise[1,4], Vincenzo Antignani[1], Tristan A. Hayes[1,2], Christopher B. Lawrence[1,2], Brett M. Tyler[4,5]. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4)

Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 5) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Fungal infections have become an increasingly significant problem for immunocompromised individuals, transplant recipients, the elderly, several cases involving healthy individuals. There is a significant growth in incidences of morbidity and mortality associated with medically important fungi, specifically Aspergillus species. Aspergillus fumigatus virulence has been attributed to production of pigments, adhesins on the surface of the cell wall, secreted proteases, and mycotoxins. Current treatments consist of oral corticosteroids, antifungal medications, and/or surgery to remove aspergillomas. Many of these treatments have substantial shortcomings. Detection and diagnosis is also weighty problem as most clinical tests take weeks for results allowing the infection to proceed. Appropriately, the paradigm for human fungal interactions has been focused on the host deficiencies mediating virulence of opportunistic pathogenic fungi. There has been substantial progress in identifying and characterizing secreted proteins (effectors) from bacterial, oomycete, and fungal plant pathogens. A subset of these effector proteins are able to enter host cells and modulate host intracellular functions. Using our bioinformatics pipeline we have been able to identify a family of secreted proteins from A. fumigatus sharing a conserved N-terminal RXLR-like motif. We found this family is expanded amongst primary fungal pathogens. The RXLR and RXLR-like motifs from known intracellular effectors of plant pathogenic and mutualistic oomycetes and fungi have been shown to facilitate effector entry into plant cells via binding external phosphatidylinositol-3-phosphate (PI3P). Here we describe AF2, a candidate effector from A. fumigatus that contains a N-terminal RxLR-like motif. Through the use of confocal microscopy and flow cytometry we show AF2 is rapidly able to enter several primary and immortalized mammalian cell lines. Through the use of isothermal titration calorimetry and liposome binding assays we show AF2 has nanomolar binding affinity for PI3P, and does not bind other mono or poly-PIPs that we have tested thus far. Based on our bioinformatics and biochemical analysis we postulate AF2 is a secreted effector protein capable of rapidly translocating into mammalian cells. We will present our latest findings on the physiological relevance of AF2.

484. A role for PalH-mediated signal transduction in *A. fumigatus* virulence and cell wall integrity: An exploitable target for combination therapy? M. Bertuzzi[1], C.M. Grice[1], L. Alcazar-Fuoli[2], A.M. Calcagno-Pizarelli[1], J. Kalchschmidt[1], S. Gill[1], K. Fox[1], A. Cheverton[1], Hong Liu[3], V. Valiante[4], E.A. Espeso[5], S.G Filler[3], A. Brakhage[4], E.M. Bignell[1]. 1) Centre for Molecular Bacteriology & Infection , Imperial College London , London (UK); 2) Mycology Reference laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid (Spain); 3) David Geffen School of Medicine at UCLA, Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center (USA); 4) Leibniz-Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Molecular and Applied Microbiology, Jena (Germany); 5) Dept. of Cellular and Molecular Biology, Aspergillus Molecular Genetics Unit, Centro de Investigaciones Biológicas (C.S.I.C.), Madrid (Spain).

Adaptation to host-imposed stress is a crucial requirement for persistence of *Aspergillus fumigatus* in the mammalian lung. In *Aspergillus* species, PacC signalling promotes tolerance of alkaline environments via signal-dependent proteolytic processing of the transcription factor PacC. The aim of this study was to test the requirement for *A. fumigatus* PalH during infection and to decipher its role in PacC-mediated signalling. The role of PalH in alkaline-mediated PacC processing was tested using electrophoretic mobility shift assay, and *A. fumigatus* virulence was examined in a murine neutropenic model of pulmonary aspergillosis. To probe the mechanistic basis of PalH-mediated signalling, we utilised a split-ubiquitin Membrane Yeast Two-Hybrid (MYTH) assay to assess protein interactions amongst candidate *A. fumigatus* signalling proteins of this pathway. *A. fumigatus* isolates expressing epitope-tagged PalH protein were constructed to assess the relevance of PalH oligomerisation. Analysis of PacC processing identified the requirement for PalH to initiate alkaline-mediated PacC signalling. A *DpalH* mutant is somewhat sensitive to alkaline pH, and attenuated for virulence in a murine model of pulmonary aspergillosis. The mutant is also sensitive to cell wall-perturbing agents, and in the presence of the cell wall-active antifungal caspofungin undergoes extensive hyphal branching and ballooning compared to the parental and reconstituted strains. In the absence of PalH *A. fumigatus*-mediated damage of epithelial cells is abrogated *in vitro*. By using a MYTH assay a significant interaction between *A. fumigatus* PalH and PalF was detected in *Saccharomyces cerevisiae*. In *A. fumigatus* PalH-mediated PacC signalling, likely implemented in a (PalF) arrestin-like manner, commands a central role in the expression of virulence-determining functions. The impairment of PacC signalling exerts a synergistically inhibitory effect upon fungal viability in the presence of cell wall-active antifungal drugs and therefore represents an attractive target for the development of novel antifungal mono- and combination therapies. Our results support a scenario whereby PalH is an oligomerising receptor, responsive to extracellular pH, and required for virulence and echinocandin tolerance. Future studies will focus upon the mechanism of PalH-mediated pH sensing.

485. Aspergillus fumigatus trehalose-6-phosphate regulates innate immune responses and virulence through modulation of fungal cell wall composition. Srisombat Puttikamonkul[2], Vishu K. Amanianda[3], Jean-Paul Latge[3], Kelly M. Shepardson[2], John R. Perfect[4], Nora Grahl[2], Bridget M. Barker[2], Robert A. Cramer[1]. 1) Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Immunology and Infectious Diseases, Montana State University; 3) Unite des Aspergillus, Institut Pasteur; 4) Medicine, Division of Infectious Diseases, Duke University Medical Center.

Mechanism(s) behind the attenuated fungal virulence of trehalose biosynthesis pathway mutants are not fully understood. We observed previously that TPS2/OrlA, a key enzyme in TPS1/TPS2 trehalose biosynthesis is required for cell wall integrity and fungal virulence in A. fumigatus. In this study, we tested the hypothesis that the significant in vivo attenuated virulence and in vitro impaired cell wall integrity of DorlA is due to accumulation of Trehalose-6-Phosphate (T6P). Our data suggest that the mechanism behind the attenuated virulence of the A. fumigatus TPS2 null mutant, DorlA, in a murine model of X-linked chronic granulomatous disease (X-CGD) is mediated by an increased susceptibility of DorlA to polymorphonuclear leukocyte (PMN) killing. In the absence of PMNs in the xCGD murine model, DorlA exhibited restored fungal burden and virulence similar to wild-type inoculated animals. Null mutations in putative trehalose biosynthesis proteins TslA and TslB in the DorlA background were able to ameliorate T6P accumulation and restore cell wall integrity and virulence strongly suggesting that accumulation of T6P is the key factor associated with DorlA virulence. Our results identify a previously unknown mechanism of immune modulation by the fungal carbohydrate metabolite T6P that has significant implications for targeting trehalose biosynthesis as an antifungal drug target.

486. Fungal lipoxygenases: a novel instigator of asthma? Gregory J. Fischer[1], Katharyn Affeldt[3], Erwin Berthier[2], Nancy P. Keller[1,2,3]. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Statement of Purpose: Fungi have long been associated with asthmatic diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown. We propose that fungal lipoxygenase enzymes and their eicosanoid products are involved in asthmatic diseases. Human 5-lipoxygenase derived leukotrienes induce inflammation, mucus secretion, vasodilation, and bronchial constriction. We hypothesize that the fungal pathogen *Aspergillus fumigatus* is capable of secreting a 5-lipoxygenase homolog, LoxB, that participates in eicosanoid production, including leukotrienes. This secreted homolog is translocated into lung epithelial cells, participates in the production of leukotriene and other eicosanoids, and exacerbates asthmatic responses, such as bronchoconstriction. Together, this work will help delineate the role fungal products play in asthmatic diseases. **Methods:** We are

assessing fungal interactions with lung epithelial cells using a microfluidic *in-vitro* platform followed by murine asthma model research. To assess the effects of LoxB overexpression, mass spectrometry was used to identify eicosanoid oxylipins within culture supernatants. **Results:** We have identified an *Aspergillus fumigatus* lipoxygenase, LoxB, with high identity to human 5-lipoxygenase. Moreover, we have identified a motif in LoxB that may mediate entry into lung epithelial cells. To fully understand the impact of LoxB in asthma, we have developed an *Aspergillus fumigatus* strain that overexpresses LoxB. Overexpression of LoxB results in increased levels of various eicosanoids that are known to cause airway hyperresponsiveness and increased mucus production. Future work will focus on characterizing the effect these eicosanoid products have on the airway and whether fungal effector translocation result in increased leukotriene levels.

487. F-box protein 15 (Fbx15) links virulence of Aspergillus fumigatus to protein degradation and stress response. Bastian Jöhnk[1], Özgür Bayram[1], Oliver Valerius[1], Thorsten Heinekamp[2], Ilse D. Jacobsen[3], Axel A. Brakhage[2], Gerhard H. Braus[1]. 1) Institute for Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Georg August University, Göttingen, Germany; 2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI) and Friedrich Schiller University, Jena, Germany; 3) Department for Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology (HKI) and Friedrich Schiller University, Jena, Germany.

Rapid adaptation to a versatile host represents a challenge for the opportunistic human pathogen *Aspergillus fumigatus* for successful infection. F-box proteins are the adaptor subunits of E3 SCF (Skp1 cullin-1 F-box protein) ubiquitin ligases. They recognize target proteins, which are marked by the SCF complex for degradation in the 26S proteasome. Here we have identified Fbx15 as an F-box protein, which links *A. fumigatus* virulence to protein degradation. *A. fumigatus* deletion strains which have lost *fbx15* are unable to infect immunocompromised mice in a murine model of invasive aspergillosis. Fbx15 is required for growth during stress including increased temperature, oxidative stress and amino acid starvation. Fbx15 is also required for controlling the synthesis of the antiphagocytic gliotoxin. Fbx15 interacts in the nucleus with the linker protein Skp1/SkpA suggesting that SCF^{Fbx15} primarily targets nuclear proteins. Four nuclear subunits of the COP9 sigalosome are putative Fbx15 interaction partners. We propose an interdependent stabilization of Fbx15 and the COP9 sigalosome, which is required to link protein degradation and stress response to virulence.

488. The sfp-type phosphopantetheinyl transferase, PPTA, is critical for the virulence of *Aspergillus fumigatus*. A. E. Johns, P. A. Warn, P. Bowyer, M. J. Bromley. Inflammation and repair, Univ. of Manchester, Manchester, United Kingdom.

Aspergillus fumigatus is the leading cause of invasive aspergillosis (IA), a fungal disease which is increasing annually on a global scale. IA poses as a common threat to patients with a weakened immune response due to disorders such as leukaemia, HIV, AIDS and also persons undergoing chemotherapy treatments. The ability of *A. fumigatus* to produce a wide array of secondary metabolites is thought to contribute to the pathogenicity of this organism. We have identified an enzyme, PPTA that plays a key role in secondary metabolism in *A. fumigatus*. PPTA is a sfp-type phosphopantetheinyl transferase and is required to activate non-ribosomal peptide synthases, polyketide synthases and a protein required for lysine biosynthesis aminoadipate reductase (AARA). Disruption of pptA prevents the production of most secondary metabolites and renders the fungus avirulent in both insect and murine infection models. To investigate which aspects of pptA activity are essential to virulence a series of knock out mutant strains were generated; DaarA, DpksP and DsidA. These genes play a vital role in lysine, melanin and siderophore biosynthesis pathways respectively. The sidA gene proved vital to virulence in the insect model whereas the DaarA and DpksP mutants were unaffected. The pathogenicity of both the pptA and sidA knock out strains was restored by co-injecting larvae with iron. We postulate that, at least in the larval model, it is PPTAs role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of *A. fumigatus*.

489. Characterization of effectors of the barley pathogen *Rhynchosporium commune*. Daniel Penselin, Wolfgang Knogge. Stress and Developmental Biology, Institute of Plant Biochemistry, Halle, Germany.

R. commune is the causal agent of barley leaf scald. This disease is a persistent threat and widespread in particular in cool and moist barley-growing areas of the world. Yield losses as high as 35-40% have been reported, but a yield loss of only 5% may already lead 2012 to an economic loss of >700 Mio € in Europe. *R. commune* colonizes the leaves of its host plants by growing beneath the cuticle, mainly in the pectic layer of the outer epidermis cell walls, without directly contacting the plant plasma membrane. Therefore, the fungus needs to secrete effectors to manipulate the host physiology. Previous studies have shown that three secreted necrosis-inducing proteins (NIP1, NIP2, NIP3) affect fungal virulence in a quantitative manner depending on the host genotype. NIP1 was also identified as the avirulence factor that is recognized by barley resistance gene *Rrs1*.

After obtaining the genome sequence of *R. commune* it turned out that NIP1 and NIP3 are encoded by single genes. In contrast, a small family of highly homologous *NIP2* genes was identified, precluding a simple targeted deletion strategy for further functional analysis of *NIP2*. In addition, deletion of one *NIP2* homolog affected the expression of the others. For further investigations an approach to simultaneously silence all members of the *NIP2* family is being followed using a recombination-based cloning strategy. To this end, a plasmid expressing an intron-containing hairpin RNA (ihpRNA) was constructed. Transfection of *R. commune* with the ihpRNA plasmid and qRT-PCR-based assessment of the transcriptional down-regulation of *NIP2* homologues are in progress. Establishing a gene silencing system will be of great value for future functional studies of fungal effectors involved in plant-pathogen interactions.

490. Molecular and genetic basis guiding the establishment of a mutualistic relationship between *Epichloë festucae* and perennial ryegrass. Sladana Bec, JinGe Liu, Christopher L. Schardl. Dept Plant Pathology, Univ Kentucky, Lexington, KY.

The relationship established between *Epichloë festucae* and perennial ryegrass (*Lolium perenne*) is a model system for studying mutualism between endophytes and cool season grasses. *E. festucae* colonizes all above-ground plant organs, growing by intercalary hyphal extension in elongating grass leaves. During the reproductive phase of growth, the fungus exhibits a dual nature: retaining its benign endophytic growth and seed transmission, or forming external stromata and suppressing seed production on affected tillers. From our previous work regarding the genes involved in the switch between benign plant colonization and formation of stromata, we have identified a number of genes encoding small secreted proteins (*ssp*) that are highly up-regulated in benign infected inflorescences. Two of those genes, *sspB*, and *sspX*, are located in a subtelomeric region, and preliminary evidence suggests that they may play a role in host specificity. Although *E. festucae* is reported to be compatible with two related host species, *L. perenne* and *Lolium pratense* (meadow fescue), strains generated from a series of crosses and backcrosses showed a range of compatibility with *L. perenne*, but consistently were compatible with *L. pratense*. One such strain, E2368, had low compatibility with *L. perenne*, whereas a subculture (variant E4844) showed improved compatibility with this host. Genomes of E4844 and E2368 were compared, revealing that the variant had lost the subtelomeric region containing *sspB* and *sspX*. The possible roles of *sspB* and *sspX*, and of other gene losses and genomic changes in the variant, are under investigation. Also, the parents and full siblings of strain E2368 are being tested for SNPs segregating for phenotypes related to the establishment of stable mutualistic symbioses with *L. perenne*. The set of progeny strains has been screened for the establishment of host specificity with perennial ryegrass, and is slated for

Illumina genome sequencing and subsequent bulk segregant analysis to identify SNP markers correlated with host-specificity phenotypes.

491. *Puccinia graminis* and *Brachypodium distachyon*: contrasting profiles of host-pathogen incompatibility. Melania Figueroa[1], Sergei Filichkin[1], Sean Gordon[2], Henry Priest[3], John Vogel[2], David Garvin[4], Todd Mockler[3], William Pfender[1]. 1) Oregon State University, Corvallis, OR; 2) USDA-ARS, WRRC, Albany, CA; 3) Donald Danforth Plant Science Center, St. Louis, MO; 4) USDA-ARS, PSRU, St. Paul, MN.

The causal agent of stem rust, *Puccinia graminis*, is a devastating pathogen that affects the production of cereals and temperate-zone grasses. Some important examples of *P. graminis* and their typical hosts are *P. graminis* f. sp. *tritici* (*Pg-tr*) on wheat and barley, *P. graminis* f. sp. *lolii* (*Pg-lo*) on perennial ryegrass and tall fescue, and *P. graminis* f. sp. *phlei-pratensis* (*Pg-pp*) on timothy grass. The biological and evolutionary attributes of *Brachypodium distachyon* have led to its development as a model to study cereals and grasses. To assess the applicability of *Brachypodium* to investigate non-host resistance to stem rust, disease severity caused by *Pg-tr*, *Pg-pp* and *Pg-lo* was evaluated across a collection of *Brachypodium* inbred lines. The different fungal isolate/*Brachypodium* line combinations demonstrated significant variation in stem rust resistance and revealed the contrasting pathogenic/virulent characteristics among these stem rust isolates. Given the distinct phenotypes obtained when inoculating with *Pg-tr*, *Pg-lo* or *Pg-pp*, *Brachypodium* line Bd1-1 was selected for further analyses. Histological analysis of the early infection events (first 68 h of infection) indicated that *Pg-lo* and *Pg-pp* are more efficient than *Pg-tr* in establishing a biotrophic interaction, and that Bd1-1 exhibits pre-haustorial resistance to *Pg-tr* and post-haustorial resistance to *Pg-lo* and *Pg-pp*. A comparative transcriptome analysis (RNA-Seq) of the early responses of Bd1-1 to *Pg-lo*, *Pg-pp* and *Pg-tr* was performed. Gene expression profiles were determined to capture the transcriptional events in response to 1) appressorium formation (12 hpi, hours post-inoculation), and 2) fungal penetration (18 hpi) and initial stages of fungal growth in the plant mesophyll for each fungal isolate. The data show distinctive profiles for each fungal isolate/*Brachypodium* combination. Our results demonstrate a significant transcriptional re-programming that leads to the activation of early plant defenses associated with quantitative resistance (i.e., phenylpropanoid pathway, cytochrome P450s, and different types of transcription factors). Additionally, several receptor-like proteins and uncharacterized proteins were identified as putative players in pathogen recognition.

492. *Magnaporthe oryzae* has evolved two distinct mechanisms of effector secretion for biotrophic invasion of rice. Martha C. Giraldo[1], Yasin F. Dagdas[2], Yogesh K. Gupta[2], Thomas A. Mentlak[2,4], Mihwa Yi[1], Hiromasa Saitoh[3], Ryohei Terauchi[3], Nicholas J. Talbot[2], Barbara Valent[1]. 1) Plant Pathology, Kansas State University, Manhattan, KS. USA; 2) School of Biosciences, University of Exeter, EX4 4QD, UK; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003 Japan; 4) Cambridge Consultants Ltd, Cambridge, CB4 0DW, U.K.

Pathogens secrete effector proteins into host tissue to suppress immunity and cause disease. Pathogenic bacteria have evolved several distinct secretion systems to target specific effector proteins during pathogenesis, but it was not previously known if fungal pathogens require different secretory mechanisms. We present evidence that the blast fungus *Magnaporthe oryzae* possesses distinct secretion systems for delivering effector proteins during biotrophic invasion of rice cells. *M. oryzae* secretes cytoplasmic effectors targeted for delivery inside rice cells and apoplastic effectors targeted to the extracellular space. Cytoplasmic effectors preferentially accumulate in the biotrophic interfacial complex (BIC), a novel in planta structure located beside the tip of the initially filamentous invasive hypha and then remaining next to the first differentiated bulbous invasive hypha cell. In contrast, apoplastic effectors remain in the extracellular compartment uniformly surrounding the invasive hypha inside the invaded cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A (BFA) treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors. Fluorescence Recovery After Photobleaching experiments confirmed that cytoplasmic effectors continued to accumulate in BICs in the presence of BFA. Analysis of mutants showed that the BIC is associated with a novel form of secretion involving exocyst components, Exo70 and Sec5, and the t-SNARE Sso1, which are required for efficient delivery of effectors into plant cells and are critical for pathogenicity. By contrast, effectors which function between the fungal cell wall and plant plasma membrane are secreted from invasive hyphae to the apoplast by the ER-Golgi secretory pathway conserved in eukaryotes. We propose a model for the distinct secretion systems that the rice blast fungus has evolved to achieve tissue invasion.

493. Trichoderma rhizosphere's competency, endophytism and plant communication: A molecular approach. Artemio Mendoza[1], Johanna Steyaert[1], Natalia Guazzone[1], Maria Fernanda Nieto-Jacobo[1], Mark Braithwaite[1], Robert Lawry[1], Damian Bienkowski[1], Christopher Brown[2], Kirstin MacLean[1], Robert Hill[1], Alison Stewart[1]. 1) Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; 2) Biochemistry Department and Genetics Otago, University of Otago, New Zealand.

Establishment of root symbiosis is one the key drivers of biocontrol success for members of the fungal genus *Trichoderma*. This root symbiosis is described as a two-step process, whereby *Trichoderma* species colonise the soil surrounding the root (rhizosphere) and then penetrate the root tissue and establish an endophytic relationship. The ability to colonise and then proliferate over time within the rhizosphere is termed rhizosphere competence (RC). There have been numerous reports of *Trichoderma* biocontrol strains which persist within the rhizosphere for the growing season of the crop plant. Our results strongly suggest that RC is widespread among members of the genus *Trichoderma* and that RC interactions are strain and host plant specific. For endophytes and their host plants to maintain a mutualistic relationship requires a constant molecular dialogue between the organisms involved. For example, the fungal-derived phytohormone, indole acetic acid (IAA), plays an important role in signalling between *Trichoderma* and the model plant *Arabidopsis thaliana*. There are however, additional, currently unknown, chemical signals which may be even more important for a positive interaction between *Trichoderma* and plants. By using a soil-maize-*Trichoderma* as a model system in in situ sterile conditions we are currently analysing the RC and endophytism transcriptomes of two *Trichoderma* species: *T. virens* and *T. atroviride*. Using a combination of bioinformatics, quantitative RT-PCR (for stage specific genetic markers from *Trichoderma*) and fluoro-labelled *Trichoderma* strains we are currently identifying and analysing promising *Trichoderma* candidates involved in endophytism and RC. A comprehensive panorama of the *Trichoderma*-soil-plant interaction will be discussed in this conference.

494. *Ustilago bromivora* - *Brachypodium distachyon*: a novel pathosystem. Franziska Rabe, Regine Kahmann, Armin Djamei. Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

The *Ustilago maydis* - *Zea mays* pathosystem is a well established model system to investigate basic principles of biotrophic plant-pathogen interactions. However, due to the long generation time, space requirements, and difficulties in transformation of maize studying the plant side is demanding. Recently, it has been shown that the yet uncharacterized smut fungus *Ustilago bromivora* infects *Brachypodium distachyon*, a model grass species. Short generation time, small size, sequenced diploid genome, and accessible reverse genetics make this monocot highly suitable for the analysis of biotrophic interactions with focus on the pathogen as well as the plant side. The primary goal of this study is therefore the characterization of *U. bromivora* and the interaction with its host in order to evaluate the suitability of the *U. bromivora* - *B. distachyon* interaction as a new host-pathogen model system.

We could show that haploid *U. bromivora* strains obtained after spore germination can be cultivated and transformed with self-replicating plasmids used for *U. maydis* transformation. A hallmark of smut fungi is that the pathogenic form is the dikaryon which arises after mating of compatible haploids. Haploid cells are produced when diploid spores germinate and undergo meiosis. Usually after germination of a single teliospore, cells with compatible

mating types can be isolated. After germination of *U. bromivora* spores (kindly provided by T. Marcel, INRA-AgroParisTech, France) all haploid progenies were of the *a1* mating type. This suggests a mating type bias where the *a2* mating type might be linked to a deleterious recessive allele making the isolation of strains harboring this mating type under laboratory conditions impossible. Hence, the genomes of several strains harboring the *a1* mating type and of diploid spore material containing both mating types were sequenced via an Illumina-Next-Generation Sequencing approach. Based on these sequences we plan to reconstruct the *a2* mating type and to generate a strain containing this mating type as well as a solopathogenic haploid strain.

495. T-DNA mediated insertional mutagenesis: evidence of a new gene implied in the early phase of pathogenic development of *Botrytis cinerea*. Nathalie Poussereau[1*], Eytham Souibguy[1], Marie-Pascale Latorse[2], Geneviève Billon-Grand[1], Cindy Dieryck[1], Vincent Girard[1], Adeline Simon[3], Muriel Viaud[3], Julia Schumacher[4], Paul Tudzinsky[4]. 1) Unité Mixte CNRS BayerSAS, Université LYON I, 14 impasse Pierre Baizet, BP 99163, 69263, Lyon cedex09, France; 2) Centre de Recherche Bayer SAS 14 impasse Pierre Baizet, BP 99163, 69263, Lyon cedex09, France; 3) BIOGER, INRA Versailles, route de Saint Cyr, 78026, Versailles France; 4) Institut fûr Biologie and Biotechnolgie der Pflanzen, West.Wihelms-universitât, Hindenburgplatz 55, 48143 Mûnster, Germany.

A collection of mutants of the grey mould fungus Botrytis cinerea has been constructed in order to provide the support for the identification of both fungal functions that are essential for the pathogenic development and/or plant defence traits raised in the host plant. A random insertional mutagenesis strategy based on the Agrobacterium tumefaciens-mediated transformation (ATMT) is used to enlarge an existing mutant library (2367 lines, Giesbert et al. 2011). 2144 additional T-DNA integrated transformants have been generated. The insertion sites of the T-DNA are being determined using TAIL-PCR and capacity to infect the host plant is assayed. These data are organized into a genome-orientated database of tagged genes and will be soon available for the scientific community. One exploitation of this mutant library focuses on the characterization of mutants whose parasitic development in planta is hampered. We present here an example of the study of a new gene encoding a DnaJ domain protein. The T-DNA mutant exhibited a drastic alteration of the infectious process on bean leaves. Deletion of the studied gene confirmed this phenotype and revealed that colonization process was also altered on different host plants. A defect in penetration and an abnormal infection cushion formation were registered. A dramatic reduced conidiation and an abnormal hyphal morphology were also observed. Resistance/sensitivity to ROS, formation of ROS, organic acids and cell wall degrading enzymes secretion were investigated. Finally, proteomic analyses are currently developed in order to attribute a function to this gene.

496. The NADPH Oxidase Complexes in Botrytis cinerea. Ulrike Siegmund, Jens Heller, Sabine Giesbert, Paul Tudzynski. IBBP, WWU Muenster, Muenster, Germany.

Reactive oxygen species (ROS) are generated in all aerobic environments and therefore play a major role for many organisms depending on oxygen. For example they act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens (Takemoto et al., 2007). One good example is the oxidative burst; plants rapidly produce large amounts of ROS as the first defense reaction towards pathogen attacks. NADPH oxidases (Nox) are the most common enzymatic system to produce these ROS. Nox are enzyme complexes, which transport electrons through biological membranes and therewith reduce oxygen to superoxide. In fungi they are shown to be involved in differentiation processes and pathogenicity and are therewith in our focus to gain insights into plant - fungi interactions. In the phytopathogenic fungus Botrytis cinerea two NADPH oxidases (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified (Segmueller et al., 2008). Besides their involvement in pathogenicity and sclerotia production, deletion studies have revealed that BcNoxA and BcNoxR are also involved in hyphal germling fusions (Roca and Weichert et al., 2011). Recent analyses show a localization of the catalytical subunits BcNoxA and BcNoxB to the ER and partly to the plasma membrane of hyphae, while the regulator BcNoxR is localized in vesicles and at the hyphal tips. Nox are multi-enzyme complexes, whose regulatory process and the participating proteins are well described in mammals. However, in fungi not all components have been identified, yet. For B. cinerea interaction studies with potential candidates identified the small GTPase Rac, the GEF BcCdc24, the scaffold protein BcBem1 and the PAKs BcCla4 and BcSte20 as interacting proteins within the BcNox complex. Roca M.G. and Weichert M. et al., (2012) Fungal Biol 116(3): 379-387. Segmueller N. et al., (2008) Mol Plant Microbe Interact 21: 808-808-819. Takemoto D. et al., (2007) Fungal Genet Biol 44(11): 1065-1076.

497. A putative function of small RNAs in the plant pathogen *Botrytis cinerea*. Arne Weiberg, Ming Wang, Hailing Jin. Plat Pathology & Microbiology, UC Riverside, Riverside, CA.

Small RNAs (sRNAs) are a class of non-coding transcripts that regulate gene expression. sRNA-directed gene regulation is a common phenomenon in eukaryotes, and in fungal systems function in differentiation, genome defense, and heterochromatin formation has been described. However, it is unknown in any systems whether sRNAs play an important role in fungal pathogenicity. To study sRNAs in the plant pathogen *Botrytis cinerea* we are currently undertaken a dual approach: I) sRNA deep sequencing was performed analyzing RNA profiles of fungal in vitro culture samples and Botrytis-infected plant tissues using two host systems, the model plant *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*). The goal is to identify infection-specific *B. cinerea*-derived sRNAs (Bc-sRNAs). II) Genetic analysis of important sRNA biogenesis factors in B. cinerea is currently piloted. *B. cinerea* possesses all relevant RNAi components including two Dicer-like genes (*Bc-DCL1* and *Bc-DCL2*) and two Argonaute-like genes (*Bc-AGO1* and *Bc-AGO2*). Targeted gene disruption by homologous recombination of *Bc-DCL1* and *Bc-DCL2* led to growth retardation on artificial media and to delay of massive conidiospore production. *In planta*, no reduction in virulence was observed. However, a *dcl1dcl2* double mutant was strongly impaired in virulence and was unable to produce a set of Bc-sRNAs. Taken our observations together, it is proposed that *B. cinerea* expresses Bc-sRNAs during infection in order to regulate important processes to facilitate pathogenesis.

498. The Role of Quorum-sensing Molecules in Interactions between *Candida albicans* and its Host. Jessica C. Hargarten[1], Thomas M. Petro[2], Kenneth W. Nickerson[1], Audrey L. Atkin[1]. 1) School of Biological Sciences, University of Nebraska, Lincoln, Lincoln, NE; 2) Department of Oral Biology, University of Nebraska Medical Center, Lincoln, NE.

Candida albicans is a polymorphic fungus that is capable of causing the life threatening disease Candidiasis once it reaches the bloodstream of a susceptible host. The capability to switch between morphologies, and its ability to synthesize and secrete the quorum sensing molecule (QSM) farnesol are known virulence factor. Previously, we showed that *C. albicans* mutants that produced less farnesol are less pathogenic to mice than their parental strain in a tail vein assay. Also, oral administration of farnesol to the mice prior to infection increased mortality. In contrast, farnesol blocks the yeast to mycelia transition in vitro, which should have a protective effect. These observations pose the dilemma of finding a mechanism whereby a molecule which blocks the yeast to mycelia transition can also act as a virulence factor. We hypothesize that farnesol functions as a virulence factor by modulating the host innate immune response. Distinct *Candida* morphologies elicit different host immune responses. Both white and opaque cells stimulate leukocyte movement, but only white cells secrete a small molecular weight chemoattractant that draws the leukocyte directly towards the white cell and stimulates engulfment by mouse macrophages. The white cells are also less susceptible to killing by human macrophages and neutrophils than opaque cells, possibly due to their increased capabilities of escape once phagocytosed. The chemical identity of this chemoattractant is currently unknown, but the reason

behind its continued secretion by white cells is intriguing. One likely candidate is farnesol because opaque cells, unlike white cells, do not accumulate detectable levels of farnesol. Macrophages are capable of detecting and responding to exogenous farnesol. Earlier our group reported that farnesol stimulates the expression of both pro-inflammatory and regulatory cytokines by mouse macrophage. The production of these warning signals is an important indicator of how the body ultimately hopes to clear the infection. Others have shown that farnesol suppresses the anti-*Candida* activity of macrophages through its cytotoxic effects, thus making it all the more difficult to eliminate the fungus early in infection. Here we report the in vitro role of farnesol and other known QSM in macrophage chemotaxis and relative phagocytosis of *C. albicans*.

499. The Role of *ISW2* for *in vitro* and *in vivo* Chlamydospore Production in *Candida albicans*. Ruvini U. Pathirana[1], Dhammika H. M. L. P. Navarathna[2], David D. Roberts[2], Kenneth W. Nickerson[1]. 1) School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE; 2) Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

The production of chlamydospores is an unusual feature in the medically important opportunistic pathogen *Candida albicans* which is commonly used as an *in vitro* diagnostic tool. These thick walled spherical structures arise from a filament tip which is termed a suspensor cell. In the process of evolution, it is hard to believe that *C.albicans* makes a spore that does not contribute to its biology and thus the function of chlamydospores is of interest. Upon careful observation of the chronic stage of *C.albicans* colonization in mouse kidneys, we often find large cells similar in appearance to chlamydospores. We characterized these large cells using sucrose density gradients and compared them with *in vitro* induced chlamydospores. The *in vivo* cells had the same buoyancy and were physiologically similar to *in vitro* chlamydospores. So we hypothesized that chlamydospores may promote the persistence of these pathogens during pathogenesis, particularly in kidneys. To test the role of chlamydospores during host infection, we used the wild type strain SC5314 and created a *ISW2* knock out mutant. An *ISW2* knock out had been reported to be completely abolish chlamydospore formation. We found that the *ISW2* mutant had significantly reduced virulence in mouse model of disseminated candidiasis and also failed to induce chlamydospores in mouse kidneys during pathogenesis . *In vitro* studies confirm the ability of these mutants for normal filamentous growth, but they failed to produce typical chlamydospores from suspensor cells. However, after three weeks they produced chlamydospore-like structures that differed from normal chlamydospore production by the complete absence of suspensor cells. As an essential ATP dependent chromatin remodeling factor in yeasts, *ISW2* affects the regulation of transcription, recombination, and DNA repair. Our findings suggest that *ISW2* may also down regulate the genes for suspensor cell formation but not the genes for chlamydospore formation indicating that these are two independent processes. Further, our investigation into *in vivo* role of chlamydospores and suspensor cells suggest that *ISW2* could be a future drug target. Further studies on gene regulation by *ISW2* in *C.albicans* will be paramount to our understanding of development and regulatory steps for chlamydospore formation and their contribution to host infection.

500. Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections. Joanna Potrykus[1], David Stead[2], Dagmar S Urgast[3], Donna MacCallum[1], Andrea Raab[3], Jörg Feldmann[3], Alistair JP Brown[1]. 1) Aberdeen Fungal Group, University of Aberdeen, Aberdeen, United Kingdom; 2) Aberdeen Proteomics, University of Aberdeen, Aberdeen, United Kingdom; 3) Trace Element Speciation Laboratory, University of Aberdeen, Aberdeen, United Kingdom.

Iron is a vital micronutrient that can limit the growth and virulence of many microbial pathogens. Here we show, that in the murine model of disseminated candidiasis, the dynamics of iron availability are driven by a complex interplay of localized and systemic events. As the infection progresses in the kidney, *Candida albicans* responds by broadening its repertoire of iron acquisition strategies from non-heme iron (*FTR1*-dependent) to heme-iron acquisition (*HMX1*-dependent), as demonstrated *in situ* by laser capture microdissection, RNA amplification and qRT-PCR. This suggested changes in iron availability in the vicinity of fungus. This was confirmed by ^{56}Fe iron distribution mapping in infected tissues via laser ablation-ICP-MS, which revealed distinct iron exclusion zones around the lesions. These exclusion zones correlated with the immune infiltrates encircling the fungal mass, and were associated with elevated concentrations of murine heme oxygenase (HO-1) circumventing the lesions. Also, MALDI Imaging revealed an increase in heme and hemoglobin alpha levels in the infected tissue, with their distribution roughly corresponding to that of ^{56}Fe. Paradoxically, whilst iron was excluded from lesions, there was a significant increase in the levels of iron in the kidneys of infected animals. This iron appeared tissue bound, was concentrated away from the fungal exclusion zones, and was accompanied by increased levels of ferritin and HO-2. This iron accumulation in the kidney correlated with defects in red pulp macrophage function and red blood cell recycling in the spleen, brought about by the fungal infection. Significantly, this effect could be replicated by selective chemical ablation of splenic red pulp macrophages by clodronate. Collectively, our data indicate that systemic events shape micronutrient availability within local tissue environments during fungal infection. The infection attenuates the functionality of splenic red pulp macrophages leading to elevated renal involvement in systemic iron homeostasis and increased renal iron loading. Simultaneously, localized nutrient immunity limits iron availability around foci of fungal infection in the kidney. In response, the fungus modulates its iron assimilation strategies.

501. Identification of the gut fungi in humans with nonconventional diets. Mallory Suhr, Heather Hallen-Adams. Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE.

Identification of the microorganisms that establish themselves inside and outside the human body is crucial to explore how the microbiome impacts human health. The recent Human Microbiome Project provides an initial compilation and identification of the gut microbiome ecosystem. It is well researched and understood that a large part of the gastrointestinal microbiota spans across the prokaryotic domain, but few studies have investigated the contribution of fungi to the human gut microbiome. Factors such as diet, genetics, and environment can play an influential role in explaining why differences in microbiota exist between human hosts. Expanding on work from our lab, this study examines the effect of nonconventional diets (e.g. vegetarians, vegans, gluten-free and lactose-free) on the GI tract fungi. DNA from fecal samples of healthy human subjects was isolated and fungal-specific ITS primers were used to target fungal DNA to obtain a baseline of data for gut fungi. *Candida tropicalis* and *C. albicans* were both detected, with *C. tropicalis* more prevalent. This relative abundance of *C. tropicalis* is in keeping with our earlier studies in people with conventional diets, and may be a regional phenomenon.

502. The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans. Jason Funt[1], Darren Abbey[7], Luca Issi[5], Brian Oliver[3], Theodore White[4], Reeta Rao[5], Judith Berman[6], Dawn Thompson[1], Aviv Regev[1,2]. 1) Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142; 2) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Masscahusetts Ave, Camridge, MA 02140; 3) Seattle Biomedical Research Institute, Seattle, WA; 4) School of Biological Sciences, University of Missouri at Kansas City, MS; 5) Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester MA 01609; 6) Tel Aviv University, Ramat Aviv, 69978 Israel; 7) University of Minnesota, Minneapolis MN 55455 USA.

Candida albicans is both a member of the healthy human microbiome and a major pathogen in immunocompromised individuals1. Infections are most

commonly treated with azole inhibitors of ergosterol biosynthesis. Prophylactic treatment in immuncompromised patients2,3 often leads to the development of drug resistance. Since C. albicans is diploid and lacks a complete sexual cycle, conventional genetic analysis is challenging. An alternative approach is to study the mutations that arise naturally during the evolution of drug resistance in vivo, using isolates sampled consecutively from the same patient. Studies in evolved isolates have implicated multiple mechanisms in drug resistance, but have focused on large-scale aberrations or candidate genes, and do not comprehensively chart the genetic basis of adaptation5. Here, we leveraged next-generation sequencing to systematically analyze 43 isolates from 11 oral candidiasis patients, collected sequentially at two to 16 time points per patient. Because most isolates from an individual patient were clonal, we could detect newly acquired mutations, including single-nucleotide polymorphisms (SNPs), copy-number variations and loss of heterozygosity (LOH) events. Focusing on new mutations that were both persistent within a patient and recurrent across patients, we found that LOH events were commonly associated with acquired resistance, and that persistent and recurrent point mutations in over 150 genes may be related to the complex process of adaptation to the host. Conversely, most aneuploidies were transient and did not directly correlate with changes in drug resistance. Our work sheds new light on the molecular mechanisms underlying the evolution of drug resistance and host adaptation.

503. Yeast-Hypha transition and immune recognition of *Candida albicans* influenced by defects in cell signal transduction pathways. Pankaj Mehrotra, Rebecca A Hall, Jeanette Wagener, Neil A.R. Gow. Aberdeen Fungal Group, Aberdeen.

During the infection process *C. albicans* has to respond to various stresses imposed by the host environment including oxidative and osmolarity stress generated by phagocytic cells such as macrophages and neutrophils, and also the cell wall stress agents such as exposure to caspofungin and other antifungal antibiotics. These stress responses area orchestrated through the activation of multiple stress pathways including the cAMP-PKA, several MAPK pathways and the Ca^{2+}-calcineurin pathway influence the cell wall shape and composition. We are investigating the effect of the activation or inhibition of these pathways on immune recognition mechanisms. We therefore determined the importance of the MAPK, cAMP-PKA and Ca^{2+}-calcineurin pathways on the fungal innate immune response by examining uptake, phagocytosis, and cytokine profile induced by mononuclear and polynuclear lymphocytes in response to a library of mutants in each of the above pathways under stressed and non-stressed conditions. We find that the activation and inhibition of these pathways plays a important role in remodeling of cell wall and hence the immunological profile. For example, deletion of *TPK1* and *CNA1* resulted in lower pro-inflammatory cytokine production. Immune- recognition was also affected by the exposure of *C. albicans* signaling mutants with Calcofluor-white, caspofungin , oxidative and osmotic stress and changes in temperature. These results suggest that stress signaling pathways act in a co-ordinated fashion to regulate yeast-hypha morphogenesis and the changes in the cell wall which in turn affects the immunological signature of the cell. Thus exposure to different microenvironments significantly modifies the immunological response to fungal cells, suggesting that responses to local stresses makes the fungal cell surface is a moving target for immunological surveillance.

504. GPI PbPga1 of *Paracoccidioides brasiliensis* is a surface antigen that activates macrophages and mast cells through the NFkB signaling pathway. C. X. R. Valim, L. K. Arruda, P. S. R. Coelho, C. Oliver, M. C. Jamur. Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, São Paulo, Brazil.

Paracoccidioides brasiliensis is the etiologic agent of paracoccidioidomycosis (PCM), one of the most prevalent mycosis in Latin America. *P. brasiliensis* cell wall components interact with host cells and influence the pathogenesis of PCM. PbPga1 is a GPI anchored protein that is up-regulated in the yeast pathogenic form. GPI anchored proteins are involved in cell-cell and cell-tissue adhesion and have a key role in the interaction between fungal and host cells. PbPga1 is an O-glycosylated protein that is localized on the yeast cell surface. Recombinant PbPga1 (rPbPga1) induces nitric oxide (NO) production and TNF-a release in murine peritoneal macrophages (Valim et al.*Plos One*, 2012). In the present study, rPbPga1 was able to activate NFkB in macrophage-like Raw cells that had been transfected with NFkB luciferase as well as in a reporter cell line for NFkB activation derived from RBL-2H3 mast cells. The results show that like macrophages, rPbPga1 also activates the transcription factor NFkB in mast cells. However, rPbPga1 does not activate NFAT nor is it able to induce liberation of beta hexosaminidase . The lack of beta hexosaminidase release suggests the PbPga1 is not able to activate RBL-2H3 mast cells via the high affinity IgE receptor. Mast cell activation by rPbPga1 does result in activation of the transcription factor NFkB suggesting stimulation of cytokine production. Taken together these results indicate that the surface antigen PbPga1 may play an important role in PCM pathogenesis by activating macrophages and mast cells.

505. *Cladosporium fulvum* effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. Andrea Sánchez-Vallet[1], Raspudin Saleem-Batcha[2], Anja Kombrink[1], Guido Hansen[2], Dirk-Jan Valkenburg[1], Jeroen R. Mesters[2], Bart P.H.J. Thomma[1]. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Lübeck, Germany.

Successful pathogens secrete effector proteins to deregulate host immunity which is triggered upon detection of pathogen-associated molecular patterns (PAMPs). Several fungal pathogens employ LysM effectors, such as Ecp6 from *Cladosporium fulvum*, to sequester fungal cell wall-derived chitin oligomers which act as PAMP and would otherwise be recognized by host immune receptors and trigger defense responses. The mechanism by which LysM effectors scavenge chitin molecules remained unknown thus far. Based on crystal structure analysis of Ecp6, we reveal a novel mechanism for chitin binding by intrachain LysM dimerization, leading to a binding groove in which chitin is deeply buried in the effector protein. Isothermal titration calorimetry experiments show that the concerted action of two LysM domains mediates a single chitin binding event with ultra-high (pM) affinity.

506. Genotypic and phenotypic characterization of *Setosphaeria turcica* reveals population diversity and a candidate virulence gene location. Santiago Mideros[1], Chia-Lin Chung[1,3], Jesse Poland[2,4], Gillian Turgeon[1], Rebecca Nelson[1,2]. 1) Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Ithaca, NY, USA; 2) Cornell University, Dept. of Plant Breeding and Genetics, Ithaca, NY, USA; 3) National Taiwan University, Dept. of Plant Pathology and Microbiology, Taipei, Taiwan; 4) USDA-ARS, Hard Winter Wheat Genetics Research Unit, Kansas State University, Manhattan, KS, USA.

The dothideomycete maize pathogen *Setosphaeria turcica* (anamorph *Exserohilum turcicum*) causes Northern Leaf Blight, one of the most common fungal diseases of maize worldwide. Little is known about the genetic basis of virulence and aggressiveness in this pathogen, although several races have been described based on their compatibility with maize resistance genes *Ht1*, *Ht2*, *Ht3* and *HtN*. To study the genetic basis of virulence and aggressiveness, we generated a F1 population consisting of 221 monosporic progeny of a cross between a race 1 strain and a race 23N strain. Genotyping-by-sequencing (GBS) was conducted on the population and an additional 13 diverse isolates that included the parental lines. We obtained between 341,000 and 428,000 sequence tags for each of the 234 isolates. Alignment to the *S. turcica* Et28A v1.0 genomic sequence (http://genome.jgi.doe.gov/Settu1/Settu1.home.html) yielded 27,174 single nucleotide polymorphisms (SNPs) at a density of 0.63 SNPs per kb. In the 13 isolates, using 9,526 filtered SNPs, we found an average nucleotide diversity (p) of 0.297. Using 564 polymorphic markers with less than 35% missing calls, we created a high-density genetic map that resulted in 23 linkage groups and a total length of 1,686 cM. The Et28A sequence has 407 scaffolds, four scaffolds formed a single linkage group in our genetic map. The rest of the genome remains fragmented. To identify genomic regions controlling virulence

and aggressiveness, isolates were characterized for in vitro and in planta phenotypes. For the former, mycelial abundance, colony diameter, pigmentation, and sporulation were rated in replicated trials. For the latter, incubation period, primary diseased leaf area and a qualitative differential score were rated on maize near isogenic lines with and without *Ht2*. Linkage mapping identified a 54.3 kb sequence of Et28A as a robust candidate region carrying *S. turcica avrHt2*. In order to manage the vast amount of genotypic and phenotypic data a MySQL database was created.

507. The secretome is linked to virulence in the yeast pathogen Cryptococcus. Leona Campbell[1], Anna Simonin[1], Janna Ferdous[1], Matthew Padula[1], Elizabeth Harry[2], Ben Herbert[3], Dee Carter[1]. 1) School of Molecular Bioscience, University of Sydney, Sydney, N.S.W. Australia; 2) iThree Institute, University of Technology, Sydney, NSW, Australia; 3) Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia.

The disease caused by pathogenic *Cryptococcus* spp. begins after inhalation of infectious propagules leading to infection of the lung. In some cases the pathogen disseminates to the central nervous system, resulting in meningoencephalitis, which can be fatal if left untreated. However, closely related strains of *Cryptococcus gattii* and its sibling species *C. neoformans* can exhibit significantly different degrees of pathogenesis in the mammalian host. As fungi utilize absorptive nutrition producing a range of secreted degradative enzymes, and as these may invoke a host response, the fungal secretome is likely to be very important in modulating host-pathogen interactions. To investigate this the secreteome was determined for a hypovirulent and a hypervirulent strain of *C. gattii*, R272 and R265 respectively, and a virulent strain of the opportunistic pathogen *C. neoformans*, KN99a. All strains were grown under conditions designed to mimic those encountered *in vivo*. Secreted proteins were analysed using two different mass spectrometry-based techniques: 1D nanoLC-MS/MS and Imaging Mass Spectrometry (IMS). The three strains secreted significantly different protein cohorts. A total of 70 proteins were identified with 47, 13 and 22 identified from R272, R265 and KN99a respectively. Only one protein was shared by all strains, a putative glycosyl hydrolase. The secretomes of R265 and KN99a primarily included uncharacterized proteins, and bioinformatic analysis suggested these proteins contained catalytic regions with roles in carbohydrate degradation. In contrast the less virulent R272 strain secreted a more diverse set of proteins including canonical cytosolic proteins such as enolase and transaldolase. These proteins have been described as fungal allergens that bind IgE. These findings indicate that virulence and the secretome are linked in *Cryptococcus*. The secretion of proteins with a putative role in nutrient scavenging by virulent strains R265 and KN99a suggest they can source nutrients from a range of available substrates, which may allow them to exploit a wider range of ecological niches including the mammalian host. In contrast, the potentially allergenic proteins secreted by strain R272 suggest this strain triggers a more effective immune response, leading to clearance of the pathogen.

508. Post-Transcriptional Regulation of the ER Stress Response in *Cryptococcus neoformans*. Virginia E. Havel, John C. Panepinto. Microbiology and Immunology, University at Buffalo, Buffalo, NY.

Cryptococcus neoformans is one of a small number of fungi able to make the transition from ambient environmental temperatures to human core body temperature. We have previously reported that the ER stress response plays an important role during host temperature adaptation. Deletion of the RNA binding protein, Puf4, results in temperature sensitivity and increased resistance to the ER stress inducing drug tunicamycin, leading us to hypothesize that Puf4 post-transcriptionally regulates the ER stress response during host temperature adaptation. The ER stress response is initiated by the transcription factor Hac1 (Hxl1 in *C. neoformans*). Hac1 translation requires unconventional splicing of the pre-spliced *HAC1* mRNA to the translated *HAC1* mRNA at the ER surface by Ire1. Time courses measuring *HXL1* mRNA splicing during a shift to 37°C demonstrate a delay in the splicing of *HXL1* in *puf4*D when compared to wild type. The delay in *HXL1* splicing in *puf4*D results in a delayed and persistent induction of ER stress response transcripts in *puf4*D compared to wild type as measured by northern blot. We hypothesize that Puf4 is required for localization of the *HXL1* transcript to the ER outer surface where it is cleaved by Ire1, thereby promoting the induction of the ER stress response. We have also shown through EMSA analysis and RNA-immunoprecipitation experiments that Puf4 is able to bind to *ALG7* mRNA. Alg7 is involved in protein glycosylation at the ER surface and is the target of tunicamycin. Based on the observation that *puf4*D has increased resistance to tunicamycin, and results demonstrating that Puf4 is able to bind *ALG7* mRNA we hypothesize that Puf4 may regulate *ALG7* mRNA by repressing translation. In our model Puf4 has a bi-modal mechanism of regulating the ER stress response. ER stress response initiation requires Puf4-mediated localization of pre-spliced *HXL1* mRNA to the ER surface. During the attenuation phase of the ER stress response, we hypothesize that ER transcripts are translationally repressed by Puf4, resulting in attenuation of the ER stress response and allowing the cell to return to homeostasis. Despite the well-studied mechanism of unconventional splicing by Hac1 and Hac1 homologs in yeast and other model systems, this study is the first to identify a RNA binding protein potentially involved its activation.

509. A morphogenesis regulator controls cryptococcal neurotropism. Xiaorong Lin[1], Bing Zhai[1], Karen Wozniak[2], Srijana Upadhyay[1], Linqi Wang[1], Shuping Zhang[3], Floyd Wormley[2]. 1) Biology, Texas A&M University, TAMU-3258, TX; 2) Biology, the University of Texas at San Antonio, San Antonio, Texas, USA; 3) Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA.

Cryptococcus neoformans is the major causative agent of cryptococcal meningitis, a disease that is responsible for more than 600,000 deaths each year. This ubiquitous environmental pathogen enters host lungs through inhalation and typically establishes asymptomatic latent infections. However, extrapulmonary dissemination often occurs in individuals with weakened immunity and Cryptococcus has a predilection to infect the brain. Brain infections are fatal and formidable to treat due to the poor penetration of most antifungals to the brain. Unfortunately, little is known about cryptococcal factors that control its neurotropism. Here we report that a morphogenesis regulator Znf2 controls the tissue tropism of cryptococcal infection. In particular, activation of Znf2 abolishes Cryptococcus extrapulmonary dissemination and consequently leads to the absence of fatal brain infections in the inhalation infection model. Although Znf2 overexpression strains are avirulent in this animal model, these strains are capable of proliferating in the animal lungs during the early stages of infections. Histological examinations and cytokine profiling revealed that the Znf2 overexpression strain causes enhanced monocyte infiltration in the animal lungs. Consistently, the Znf2 overexpression strain stimulates pro-inflammatory host responses while suppresses deleterious Th2 host responses during early stage of infection in the pulmonary infection model. Such protective host defense responses might have prevented the extrapulmonary dissemination of Cryptococcus. In the intravenous infection model where the lung infection was bypassed and there was uniform hematogenous dissemination, the Znf2 overexpression strain showed a specific defect in the brain infection. Taken together, our data indicate that Znf2 helps polarize the host immunity towards protection and that it mediates cryptococcal tissue tropism during infection.

510. Extracellular and intracellular signaling orchestrates morphotype-transition and virulence in human pathogen *Cryptococcus neoformans*. Linqi Wang, Xiuyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, College Station, TX.

Interactions with the environment and divergent species drive the evolution of microbes. To sense and rapidly respond to these dynamic interactions, “simple” microbes developed bet-hedging social behaviors, including the construction of heterogeneous biofilm communities and transition between different morphotypes. The human fungal pathogen *Cryptococcus neoformans* can undergo morphotype transition between the yeast and the filamentous form. Most recently, we demonstrated that the zinc-finger regulator Znf2 bridges the bi-direction yeast-hypha transition and virulence in this pathogen.

One of Znf2 downstream targets is extracellular protein Cfl1. Cfl1 is a cell-wall bound adhesin and a signaling molecule when it is released. This matrix protein Cfl1 plays a similar but less prominent role than Znf2 in orchestrating morphogenesis and virulence in *C. neoformans*. Through transcriptome analyses and screening Znf2 downstream targets by overexpression, we identified an additional player in the control of morphogenesis and biofilm formation. This factor is an intracellular RNA-binding protein Pum1. As expected, Pum1 affects filamentation in a Znf2 dependent manner. However, the effect of Pum1 on morphogenesis is independent of Cfl1. The *pum1*D *cfl1*D double mutant shows a more severe defect in filamentation than either of the single mutant, indicating that Pum1 and Cfl1 act in two parallel pathways. Two of Pum1's targets, Fad1 and Fad2, form a *Cryptococcus*-specific adhesin family. Like Cfl1, these two extracellular adhesins show regulatory roles in conducting morphogenesis and virulence in *C. neoformans* and thus may be involved in extracellular signaling transduction. Our results indicate that complex regulatory cascades composed of extracellular and intracellular circuits may be responsible for mediating morphological transition in response to the cues in the environments and the host.

511. Evidence for alkaloid diversity and independent hybridization events of *Elymus* endophytes. Nikki D. Charlton[1], Juan Pan[2], Daniel G. Panaccione[3], Christopher L. Schardl[2], Carolyn A. Young[1]. 1) Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK; 2) Department of Plant Pathology, University of Kentucky, Lexington, KY; 3) Division of Plant & Soil Sciences, West Virginia University, Morgantown, WV.

The epichloae form mutualistic symbioses with cool-season grasses and have been shown to impart biotic and abiotic fitness benefits to their hosts. Endophyte-infected plants often have greater resistance to biotic stresses such as mammalian and insect herbivory due to the presence of fungal synthesized alkaloids. Four classes of bioprotective alkaloids have been described, which include ergot alkaloids, indole-diterpenes, a pyrrolopyrazine (peramine), and saturated aminopyrrolizidines (lolines). *Elymus* species, such as *Elymus canadensis* and *E. virginicus*, are cool-season bunchgrasses native to much of North America and are known to harbor *Epichloë* endophytes. Three species are able to associate with *Elymus*: the non-hybrids *Epichloë amarillans* and *Epichloë elymi* and *Epichloë canadensis*, a hybrid with *E. elymi* and *E. amarillans* ancestral progenitors. The distribution and alkaloid genotypic variation of these fungi was examined to determine endophyte variation that may provide ecological benefits to the host. Endophyte infection frequencies from natural populations and germplasm resources ranged from uninfected to highly infected. Analyses of microsatellite loci and mating type genes characterized the prevalence and distribution of hybrid and non-hybrid endophytes among and between *Elymus* populations. Overall, non-hybrids were more prevalent than hybrids in the northern region of the U.S., whereas hybrids were more ubiquitous in the southern region. Genotypic analysis based on presence and absence of key alkaloid biosynthesis genes provided information about the potential alkaloid diversity within these populations. Thirteen unique alkaloid genotypes were identified that showed variation within the *EAS* (ergot alkaloid), *LOL* (loline) and *PER* (peramine) loci that indicates some genotypes are likely to accumulate pathway intermediates. Evaluation of the mating-type idiomorphs from the hybrid *E. canadensis* indicates this species has resulted more than once through independent hybridization events thus explaining variation found among the alkaloid genes. Chemical analyses of representative endophyte-infected plants are being conducted to correlate alkaloid predictions with actual alkaloid production. Chemotype diversity will be evaluated to determine how this translates into differences in fitness and persistence of the host.

512. The functional characterization of candidate genes involved in host specialization of Zymoseptoria grass pathogens. Stephan Poppe, Petra Happel, Eva Stukenbrock. Fungal Biodiversity, Max Planck Institute Marburg, Marburg, Germany.

The ascomycete fungus Zymoseptoria tritici (synonym: Mycosphaerella graminicola) emerged as a new pathogen of cultivated wheat during crop domestication about 11.000 years ago. To understand the molecular basis of host specialization in this pathogen we have sequenced complete genomes of Z. tritici and closely related species infecting wild grasses. Evolutionary genomic analyses allowed us to identify 17 genes that show strong evidence of positive selection between Z. tritici and the closely related sister species Zymoseptoria pseudotritici. We hypothesize these evolved in a co-evolutionary arms race with different hosts. None of the genes encode proteins with known function. In this study we focus on three candidate genes Mgr80707, Mgr 89160 & Mgr 103264 and investigate their role in Z. tritici and its two closest relatives Z. pseudotritici and Z. ardabiliae during host infection. Quantitative Real time PCR experiments from the three fungal species infecting four different grass species show that the three genes are strongly up-regulated in planta and that candidate gene expression differs over a time course of 28 days supporting a role in host pathogen interaction. In addition, we show that three different host species (wheat, Elymus repens and Lolium multiflorum) differentially induce gene expression in the fungi. Confocal Laser Scanning Microscopy conducted at different time points reveals clear differences between Zymoseptoria species during infection and within host development in wheat and Brachypodium distachyon. Deletion strains for each candidate gene have been created by Agrobacterium tumefaciens mediated transformation. The single deletion of two candidate genes Mgr80707 & Mgr103264 led to a reduced virulence of Z. tritici on wheat. The deletion of the third gene Mgr89160 led to a hyper-virulence phenotype suggesting an avirulence function of the gene product. Our study confirms that genes involved in host specialization can be identified based on footprints of natural selection.

513. Diversity and Phylogeny of genus *Suillus* (Suillaceae, Boletales) from Pakistan (Asia). Samina Sarwar, Abdul Nasir Khalid. Botany, University of the Punjab, Lahore, Punjab, Pakistan.

Coniferous forests of Pakistan are rich in mycodiversity. However, only a few scientific researches have been conducted in these forests. This paper aims to document diversity of Suillus in these forests. During a survey conducted during 2008-2010, a total of thirty two (32) basidiomata were collected. Most of them were found associated with Pinus wallichiana and Abies pindrow. Only a few were found with Cedrus deodara, Populus ciliata and Quercus spp. These basidiomata were characterized morphologically as well as by molecular analysis by amplifying rDNA. Fungal specific primers ITS3 & ITS6R and ITS2 & ITS8F were used to amplify the ITS1 and ITS2 along with partially 5.8S gene. Out of these, twelve (13) different Suillus species were found. Among them two (2) species seem undescribed and three (3) as new records for Pakistan. Their Phylogenetic relationships have also been discussed.

514. *Saprolegnia* species can switch hosts to cause infection: a new insight into host pathogen interaction. Mohammad N. Sarowar[1*], A. Herbert van den Berg[1], Debbie McLaggan[1], Mark Young[2], Pieter van West[1]. 1) Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom; 2) Department of Zoology, University of Aberdeen, Aberdeen, AB24 2TZ, United Kingdom.

Saprolegnia species are destructive oomycete pathogens of many aquatic organisms and are found in all parts of the world. Phylogenetic analysis has shown that *Saprolegnia* strains isolated from different aquatic organisms have a close relationship to fish pathogenic *Saprolegnia* species. We have now demonstrated, for the first time, that *Saprolegnia* spp. can actually switch hosts. *Saprolegnia australis*, *Saprolegnia hypogyna* and 2 strains of *Saprolegnia diclina* were isolated from insects. We also collected other oomycete species, including *S. australis*, *S. ferax*, *Pythium pachycaule* and *Pythium* sp., in water of a medium to fast running river. The ITS region of all these isolates was sequenced. Four isolates collected from the aquatic insects together with isolates of *S. parasitica* (collected from salmon), *S. diclina* (collected from trout eggs) and *S. ferax* (collected from an amphibian) were tested for pathogenicity on nymphs of a stonefly (*Perla bipunctata*), Atlantic salmon eggs and frog (*Xenopus leavis*) embryos. Most of the isolates were highly pathogenic on all tested aquatic animals. These results suggest that *Saprolegnia* spp. are capable of switching host, which may be related to seasonal variation of host availability in

fresh water environments.

515. The Plant-Microbe Interfaces project: defining and understanding relationships between *Populus* and the rhizosphere microbiome. Christopher Schadt[1], Dale Pelletier[1], Timothy Tschaplinski[1], Edward Uberbacher[1], Hurst Gregory[2], E. Peter Greenberg[3], Caroline Harwood[3], Amy Schaefer[3], Rytas Vilgalys[4], Francis Martin[5], Mitchel Doktycz[1], Gerald Tuskan[1], and other PMI researchers (http://pmi.ornl.gov). 1) Bioscience Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 3) Department of Microbiology, University of Washington, Seattle, WA, USA; 4) Department of Biology, Duke University, Durham, NC, USA; 5) Institut National de la Recherche Agronomique, Nancy, FRANCE.

Microbial interactions benefit plant health by affecting nutrient uptake, hormone signaling, water and element cycling in the rhizosphere and/or conferring resistance to pathogens. The model tree species *Populus* provides an opportunity for microbiome research relevant to bioenergy, carbon-cycle research, and other ecosystem processes. In an effort to define *Populus'* microbiome, root and rhizosphere samples from *P. deltoides* in the eastern US and *P. trichocarpa* in western US were subjected rRNA pyrosequencing and an isolate collection of over 5000 bacteria and 500 fungi obtained. We show that the rhizo- and endo-sphere environments feature highly developed, diverse and to a large degree often exclusive communities of bacteria and fungi. Endophytic bacterial diversity was found to be highly variable, but on average tenfold lower than the rhizosphere, suggesting root tissues provide a distinct environment supporting relatively few species. Fungal endophytic species were more numerous, but also less than rhizosphere spp. Both fungal and bacterial rhizosphere samples showed distinct phylogenetic composition patterns compared to the more variable endophyte samples. Contrary to initial expectations, both *Populus* spp. have low natural levels of colonization by ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi, but high levels of presumed fungal endophytic taxa such as *Nectria, Mortierrella*, and members of the *Atractiellales*. Select isolates are being studied at a whole-genome level to enable comparative work on on the basis for observed symbioses. Thus far ~43 bacterial and 5 fungal isolates have been sequenced. Initial comparative genomics of these isolates suggest highly divergent physiological and molecular mechanisms of the interactions, even within rather closely related species. Efforts to understand ECM interactions have shown that host defense networks and the ability to bypass such networks through small protein and phytohormone signals has a large effect on the ability of *Populus* to form ECM relationships. *Laccaria bicolor* is able to modulate host defense response in *P. trichocarpa,* yet unable to do so in *P. deltoides*. Mycorrhizal Helper Bacteria from the genus *Pseudomonas* partially alleviate this colonization weakness through yet unknown molecular mechanisms, illustrating the value of integrated microbiome wide studies.

516. Do the fungal homologs of *Verticillium dahliae* effector Ave1 act as virulence factors? Jordi C. Boshoven[1], Melvin D. Bolton[2], Bart P.H.J. Thomma[1]. 1) Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, Netherlands; 2) Agricultural Research Service, Northern Crop Science Laboratory, US Department of Agriculture, Fargo, ND 58102.

Verticillium species cause vascular wilt disease in over 200 plant hosts, including economically important crops. In tomato, the Ve1 immune receptor confers resistance to race 1 strains of *V. dahliae*, but not to race 2. By population genome sequencing of race 1 and race 2 strains, the effector that is recognized by Ve1 was recently identified as Ave1 (**A**virulence on **Ve1** tomato). Ave1 has homology to plant natriuretic peptides that are regulators of homeostasis, and acts as a virulence factor on tomato plants that lack *Ve1* as well as on *Arabidopsis*. In addition to plants, *Ave1* homologs were also found in a few fungal pathogens, including *Fusarium oxysporum*, *Cercospora beticola* and *Colletotrichum higginsianum*, as well as in the bacterial plant pathogen *Xanthomonas axonopodis*. Co-expression of *V. dahliae Ave1* and tomato *Ve1* in *Nicotiana tabacum* resulted in the activation of a hypersensitive response. Remarkably, also co-expression of some of the *Ave1* homologs with *Ve1* activated a hypersensitive response. Here, we evaluate whether the various pathogen-derived Ave1 homologs are virulence factors. Expression of the *Ave1* homologs of *Fusarium*, *Cercospora* and *Colletotrichum* during infection on tomato, sugarbeet, and *Arabidopsis*, respectively, was analysed. To investigate the potential role of the *Ave1* homologs in virulence, a *V. dahliae Ave1* deletion mutant was complemented with the *Ave1* homologs of *Fusarium*, *Cercospora* and *Colletotrichum*, and tested for full aggressiveness on tomato. Finally, targeted gene deletion was pursued in *Fusarium*, *Cercospora* and *Colletotrichum* and the corresponding deletion strains were inoculated on tomato, sugarbeet, and *Arabidopsis*, respectively.

517. The candidate effector repertoire of closely related *Venturia* pathogens of the Maloideae revealed by whole genome sequence and RNA sequencing analyses. Cecilia Deng[1], Daniel Jones[2], Bruno Le Cam[3], Kim Plummer[2], Carl Mesarich[4], Matthew Templeton[1], Joanna Bowen[1]. 1) Plant & Food Research, Auckland, New Zealand; 2) La Trobe University, Melbourne, Australia; 3) IRHS, INRA Angers, France; 4) Wageningen University, The Netherlands.

The genus *Venturia* includes pathogens that infect members of the Rosaceae. The most widely researched of these is *V. inaequalis* that causes the disease apple scab. Related species cause disease of other woody hosts closely related to *Malus*; eg *V. pirina* infects European pear. Certain isolates that are classified as *V. inaequalis* are unable to infect *Malus* but instead infect different hosts belonging to the subfamily Maloideae, such as loquat. Host-cultivar specificity is also demonstrated by isolates of *V. inaequalis* that infect *Malus*; 17 gene-for-gene pairings between effectors (pathogen proteins that enhance disease) and resistance gene products have been identified to date. Thus the effector repertoire of isolates of *V. inaequalis* determines their cultivar specificity and most probably host specificity. Effectors have yet to be cloned from *V. inaequalis*. Draft whole genome sequences (WGS) of three *V. inaequalis* isolates (two from apple, one from loquat) and an isolate of *V. pirina* have been assembled and candidates that share the characteristics of fungal effectors (small, secreted proteins) have been identified. Of the 13333 predicted genes in the WGS of *V. inaequalis* isolated from apple, 1088 encode putatively secreted proteins identified utilising algorithms to detect secretion signals and putative cellular location. The expression (measured by transcriptome analysis) of 119 of these is up-regulated, with a false discovery rate less than 0.05 and a log-fold change greater than 2, *in planta* compared with *in vitro* at both 2 and 7 days post inoculation (dpi), 73 at 2 dpi, and 54 at 7 dpi. Of these 246, only 43 have similarities (<e-05) to genes in publicly available databases with a large proportion encoding proteins with similarities to plant cell wall-degrading enzymes. There are a further 627 genes up-regulated *in planta* that are not predicted to be secreted; these include numerous genes putatively involved in secondary metabolism. By comparing WGS of isolates with differing specificities candidate effectors determining host range have been identified; 84 are unique to *V. pirina*, 6 unique to a *V. inaequalis* isolate specific to loquat and 145 present in all the apple-infecting isolates but absent from those able to infect loquat and *V. pirina*. These candidates are currently being characterised with respect to functionality.

518. Thermodynamic characterization of RXLR and RXLR-like effectors binding to phosphatidylinositiol-phosphates. Kelly Drews[1,2], Gloria Trivitt[1,2], Tian Zhou[1,2], Samantha Taylor[4,5], Helen Clark[1,3], Brett Tyler[4,5], Shiv Kale[1]. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 5) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Microbial-host interactions encompass a wide array of components to mediate symbiosis. The study of small-secreted proteins capable of manipulating host cellular machinery is currently a core-theme of microbial-host interactions. A number of bacterial symbionts deliver these effector molecules into

cells via type three section systems, a syringe like apparatus that directly penetrates the host cell plasma membrane. Currently, there is no evidence for such translocation machinery being present or utilized by fungal and oomycete symbionts to deliver effector proteins. Several bacterial and fungal toxins utilize external glycosphingolipids to mediate translocation into cells. Upon internalization a subset of these toxins escape endosomal compartments via retrograde transport to the Golgi-endoplasmic reticulum trans network, while others are capable of flipping across the endosomal membrane. The genus Phytophthora contains a number of highly destructive plant pathogens. Comparative genomics and the sequence availability of known intracellular effectors resulted in the discovery of a highly conserved N-terminal motif RXLR-dEER that defines an expansive super family of secreted proteins. The RXLR-dEER motif of PsAvr1b, PsAvr1k, and PsAvh5 has been shown to facilitate effector entry into a plant and animal cells in the absence of any pathogen encoded machinery. Entry is believed to be mediated by binding cell surface phospholipid, phosphatidylinositiol-3-phosphate. Using isothermal titration calorimetry we have characterized the phospholipid binding properties of the RXLR effectors PsAvr1b, PiAvr3a, and several RXLR-like effectors to mono and poly phosphatidylinositol-phosphates in a variety of experimental conditions. As controls we have characterized the phospholipid binding properties of 2xFYVE and Vam7pX, two known PI3P binding domains.

519. Investigating virulence effectors in the poplar-poplar rust pathosystem. Sebastien Duplessis[1], Benjamin Petre[1], Hugo Germain[2,3], Arnaud Hecker[1], Stéphane Hacquard[1], David L Joly[4], Armand Séguin[2], Nicolas Rouhier[1]. 1) UMR1136 IAM, INRA, Champenoux, France; 2) Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada; 3) Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada; 4) Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada.

Foliar rust caused by *Melampsora larici-populina* is a major disease affecting poplar plantations throughout the world. The obligate biotrophic status of the fungus and the perennial status of the host plant, make molecular investigation of this interaction a real challenge. However, availability of both *Populus trichocarpa* and *Melampsora larici- populina* sequenced genomes has allowed for setting an emerging model pathosystem to decipher the molecular bases of disease resistance in trees and of biotrophic growth in rust fungi. Comprehensive analyses of rust transcripts encoding small secreted proteins expressed *in planta* identified putative virulence factors and we focused our attention on a few candidates that show evidence of purifying selection between paralogs. In particular, two gene families encode modular SSP with a conserved N-terminal part and a C-terminal part evolving under positive selection except highly-conserved cysteine residues. Gene family 5464 contains 13 members, homologs of *Melampsora lini* AvrP4 avirulence factors whereas H1 family gather 31 genes specific to *M. larici-populina*. Some of these genes present a transient peak of expression during the biotrophic growth of the fungus in poplar leaves, and the corresponding proteins are able to enhance bacterial growth when delivered in *Arabidopsis thaliana* from *Pseudomonas syringae* pv *tomato* DC3000. Currently, we combine a range of approaches including biochemical and structural characterization of recombinant proteins, yeast two-hybrid and pull-down assays to characterize these candidate effectors.

520. Functional analysis of *Aphanomyces euteiches* effectors, a legume root pathogen. D. Ramirez-Garces, L. Camborde, H. San Clemente, A. Cerutti, B. Dumas, E. Gaulin. LRSV UMR5546 CNRS/UPS, Castanet-Tolosan, France.

Aphanomyces euteiches is an oomycete infecting roots of various legumes species such as pea, alfalfa and the model legume *Medicago truncatula*. The genus *Aphanomyces*, which belongs to the group of Saprolegniales, is phylogenically distant from the well known *Phytophthora* genus and comprises both animal pathogen and plant pathogen species. The first genome draft of *Aphanomyces euteiches* (ATCC201684, 57 Mb) will be soon released and genome sequencing of zoo- and phytopathogen species are under progress. *A. euteiches* genome miming revealed the expansion of CRNs (Crinkling and Necrosis) genes, initially identified in *Phytophthora infestans*. These modular proteins contain a conserved N-terminal characterized by the presence of a LFLAK amino acid motif implicated in the protein translocation from the pathogen to the host cell, whereas the modular C-terminal effector domain is highly diverse. The proposed role of the CRNs effectors is to suppress plant defense or to modulate other host cell processes that increase susceptibility and enhance pathogen virulence. In *A. euteiches*, the active translocation LYLAK motif was detected, and conserved, as well as original effector subdomains, were identified. Functional studies conducted on two types of *A. euteiches* CRNS, AeCRN5 and AeCRN13, showed that both proteins are highly induced during infection of *M. truncatula* roots. *In planta* expression of both proteins revealed host nucleus localization and cell-death induction or alteration of roots architecture when expressed in plant cells. Such observations suggest that *A. euteiches* CRNs are virulence proteins exerting their function through the interaction with nuclear compounds. Latest results regarding the putative function of AeCRNs will be presented.

521. Participation of effector proteins from Trichoderma spp. in interaction with Arabidopsis thaliana. P. Guzman-Guzman[1], M.I. Aleman-Duarte[2,3], L.J. Delaye-Arredondo[3], A. Herrera-Estrella[2], V. Olmedo-Monfil[1]. 1) Biology Dept,University of Guanajuato, Guanajuato, Guanajuato, Mexico; 2) CINVESTAV, Langebio Unit, Irapuato Unit, Irapuato, Guanajuato, Mexico; 3) CINVESTAV, Irapuato Unit, Irapuato, Guanajuato, Mexico.

When a plant-pathogen interaction is established, plant activates its immune response system, recognizing pathogen's virulence factors. In plant response are involved several phytohormones. Studies on plant pathogenic processes triggered by different organisms have shown the involvement of some pathogen proteins that are capable of altering the function and structure of the host cell, facilitating their entry and affecting overall host physiology, these proteins are known as "effectors". The effectors activity inside the host takes place through conserved mechanisms of molecular interaction, such as effectors that have the RXLR translocation motif, which is highly conserved among different pathogens, directing its entry into the host cells. In plant-pathogen interactions, effectors are recognized, activating the plant response system. However, some of these molecules have been involved in the establishment of plant interactions with non-pathogenic organisms; little is known about the mechanisms controlling the establishment of this beneficial interaction. Fungi of the genus Trichoderma establish beneficial interactions with plants, promoting their growth and defense systems. To better understand this biological process, it is important to identify effector-like molecules in species like Trichoderma, and to establish their role in promoting plant development and defense systems activation. To achieve this goal, we will test the interaction of Trichoderma species with Arabidopsis thaliana, focusing on the selection and identification of effector-like molecules that are expressed during this interaction process. Additionally, we will generate null Trichoderma mutants on these effector-like candidates to evaluate their participation in the plant-fungus interaction. Until now, we have established interaction conditions, confirming the effect of the fungus presence over plant biomass production and root growth, showing interesting morphologic changes in the root system, such as a significant increase in lateral root formation. Based on conserved characteristics and bioinformatics tools, we selected 16 encoding sequences for effector-like molecules among T. virens and T. atroviride genomes, and we confirmed the differential expression of 7 of them in interaction with A. thaliana, by RT-PCR analysis.

522. The role of LysM effectors in fungal fitness. Anja Kombrink[1], Jason Rudd[2], Dirk-Jan Valkenburg[1], Bart Thomma[1]. 1) Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom.

LysM effector genes are found in the genomes of a wide range of fungal species. The encoded LysM effectors are secreted proteins that contain a varying

number of LysM domains, which are carbohydrate-binding modules. Ecp6, secreted by tomato leaf mould fungus Cladosporium fulvum, is the first characterized LysM effector. We demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as a microbial-associated molecular pattern (MAMP) and triggers immune responses upon recognition by the host. Ecp6 outcompetes plant receptors for chitin binding, and thus prevents the activation of immune responses. Many fungal genomes, including saprophytes, carry multiple LysM effector genes that share only low sequence conservation and encode a varying number of LysM domains. We speculate that fungal LysM effectors might bind different carbohydrates and exert various functions in fungal fitness. In the fungal wheat pathogen Mycosphaerella graminicola, two LysM effectors were identified. Mg3LysM, but not Mg1LysM, suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both Mg1LysM and Mg3LysM inhibit degradation of fungal hyphae by plant chitinases, revealing an additional function for LysM effectors in pathogen virulence. We recently observed that Mg1LysM binds to the bacterial cell wall constituent peptidoglycan. Similarly, a LysM effector from the saprophytic fungus Neurospora crassa showed peptidoglycan binding. We hypothesize that peptidoglycan binding by LysM effectors plays a role in the interaction of fungal species with bacterial competitors. The soil-borne fungal pathogen Verticillium dahliae contains seven LysM effectors genes of which one (Vd2LysM) is induced during tomato infection. Inoculation with two independent knock-out mutants revealed that Vd2LysM is required for full virulence of V. dahliae. However, Vd2LysM does not specifically bind chitin and does not function in a similar fashion as previous characterized LysM effectors. Thus, its function in virulence remains unclear.

523. WITHDRAWN

524. Functional analysis and localization of SnTox1, a necrotrophic effector produced by the wheat pathogen Stagonospora nodorum. Zhaohui Liu[1], Weilin Shelver[2], Justin Faris[3], Timothy Friesen[1,3]. 1) Department of Plant Pathology, North Dakota State University, Fargo, ND; 2) USDA-ARS, Biosciences Research Laboratory, Fargo, ND; 3) USDA-ARS, Northern Crop Science Laboratory, Fargo, ND.

SnTox1 is one of the necrotrophic effectors produced by the fungus Stagonospora nodorum, the causal agent of wheat Stagonospora nodorum blotch. It interacts, directly or indirectly, with the product of the wheat gene Snn1 to induce host cell death and promote disease. Previously, we showed that SnTox1, a cysteine-rich protein, triggers programmed cell death-like responses in the host and plays an important role in fungal penetration. In the present work, we are investigating the biochemical and molecular function of SnTox1 as well as its mode of action. Based on a Prosite motif search of SnTox1, multiple predicted sites including a putative chitin binding domain were targeted for site-directed mutagenesis. SnTox1 activity was significantly reduced when mutations were produced at a casein kinase II phosphorylation site and a predicted helical region where lysine residues are abundant.Using a fungal strain expressing an SnTox1-GFP fusion protein, we examined the location of the SnTox1 protein during fungal growth and infection. SnTox1 was observed in higher concentration on several fungal structures, including the surface of conidia and mycelium, hyphal septa, and hyphal tips. The accumulation of SnTox1-GFP is particularly obvious at hyphal regions where new hyphae are arising. This observation suggests a protection mechanism of SnTox1 that is similar to that of chitin binding proteins in other fungal pathogens. In planta, SnTox1 is highly expressed in the hyphopodia where the penetration is initiated, providing further evidence that SnTox1 plays a role in penetration. The cellular localization of SnTox1 was also investigated using fluorescine labeled SnTox1 in combination with cytological methods and preliminary data has indicated that SnTox1is likely not internalized into mesophyll cells but remains in the apoplast. Interestingly, SnTox1 is able to induce host cell death by directly spraying onto the leaf surface of sensitive lines. We are currently investigating if SnTox1 is transported through epidermal cell layer.

525. Host-targeting protein 3 (SpHtp3) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a pH and tyrosine O-sulfate-dependent manner. Lars Löbach[1*], Stephan Wawra[2], Irene de Bruijn[2], Aleksandra Toloczko[2], Tim Rasmussen[3], Christopher Secombes[1], Pieter van West[2]. 1) Scottish Fish Immunology Research Centre, University of Aberdeen, School of Biological Sciences, Aberdeen, Scotland, UK; 2) Aberdeen Oomycete Laboratory, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK; 3) University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK.

The success of eukaryotic oomycete pathogens depends largely on effector proteins, molecules which manipulate or interfere with host defence mechanisms in the extracellular space or inside their host cells. One economical important oomycete parasite is the fish pathogen *Saprolegnia parasitica*, which is the causal agent of the disease Saprolegniosis. *S. parasitica* is responsible for devastating losses in the aquaculture industry worldwide. In order to effectively fight any pathogen it is crucial to understand the key molecular mechanisms that lead to the disease. With the focus on putative effector proteins we screened the genome of *S. parasitica* in the present study for potential effector candidates. Analysis identified a novel putative secreted *S. parasitica* effector protein, which we named host-targeting protein 3 (SpHtp3). Gene expression analyses showed that mRNA levels of *SpHtp3* are highest in mycelium, sporulating mycelium and during the later stages of infection. Recombinant SpHtp3 was able to translocate specifically into fish cells in a tyrosine O-sulfate and pH dependent manner. SpHtp3 was found in vesicular structures inside fish cells and was released from these upon infection of the cells with *S. parasitica*. Interestingly, SpHtp3 possesses an N-terminal RTLR tetra-peptide sequence at a similar location as found in RxLR-effectors from plant pathogenic oomycetes. However, this RTLR-sequence was not required for the fish cell translocation property of SpHtp3. These findings suggest that SpHtp3 from *S. parasitica* is a novel intracellular protein that might play an important role in Saprolegniosis.

526. *Ave1*-like orthologs in *Venturia*: another expanded effector family emerges. Adam Taranto[1], Daniel Jones[1], Jason Shiller[1], Shakira Johnson[1], Nathan Hall[1], Ira Cooke[1], Gert Talbo[1], Carl Mesarich[2], Bart Thomma[2], Jordi Boshoven[2], Joanna Bowen[3], Cecilia Deng[3], Matthew Templeton[3], Kim M. Plummer[1]. 1) Dept Botany, La Trobe Univ, Melbourne, Victoria, Australia; 2) Laboratory of Phytopathology, Wageningen University, The Netherlands; 3) Plant & Food Research, Auckland, New Zealand.

Effectors are secreted by pathogens to modify plant physiology and establish disease. Plant immune receptors have evolved to recognise effectors and counter attack with defence responses. Most fungal effectors are lineage-specific, i.e. they are unique to a species, or to physiological races within a species. The availability of many whole genome sequences has revealed that some effectors are found in a discontinuous distribution within the fungal kingdom; a few phytopathogenic fungi (*Colletotrichum higginsianum, Cercospora beticola, Fusarium oxysporum*) possess an ortholog of *Ave1* from *Verticillium dahliae*, an effector that activates Ve1-mediated resistance in tomato. A subset of these orthologs were shown to activate Ve1-mediated resistance in tomato. Unusually, Ave1 also shares similarity to an ortholog in the phytopathogenic bacterium *Xanthomonas axonopodis*, as well as to a widespread family of plant natriuretic peptides and expansins, involved in plant homeostasis and plant cell wall modification (de Jonge & van Esse et al. 2012). We have identified an expanded *Ave1*-like gene family in apple and pear scab fungi, *Venturia inaequalis* and *V. pirina*. These species also have expanded gene families with similarity to the *Leptosphaeria maculans* effector *AvrLm6*. *V. pirina* has 14 unique hits (best,$1.43e^{-18}$) to *VdAve1*. *V. inaequalis* has 17 unique hits (best,$1.07e^{-22}$) to *VdAve1*. The distribution of *Ave1* orthologs is suggestive of one or more cross-kingdom gene transfer events. We are characterising *Venturia Ave1*-like genes to investigate the mode of gene multiplication; seek evidence of horizontal gene transfer; and determine the role

of *Ave1*-like genes in pathogenicity. *Ave1*-like genes from non-*Venturia* fungi (and the bacterial gene) do not contain predicted introns, however, several (not all) *V. inaequalis Ave1*-like genes are predicted to contain introns. Codon usage bias among fungal, plant, and bacterial *Ave1* orthologs, are being compared with the aim of inferring the kingdom of origin of the *Venturia Ave1* orthologs. At least two *ViAve1* orthologs are up-regulated during infection of apple. To determine whether the *Venturia Ave1* proteins also activate a Ve1-mediated hypersensitive response, each has been co-expressed with tomato *Ve1* in *Nicotiana tabacum*, using an *Agrobacterium tumefaciens*-mediated transient transformation assay.

527. Identification of targets of mycorrhizal effector proteins in planta. Natalia Requena, Carolin Heck, Ruben Betz. Molecular Phytopathology, Karlsruhe Institute of Technology, Karlsruhe, Germany.

Plant roots are constantly approached by a myriad of microorganisms and are thus challenged to recognize friends from foes. Most plant roots engage in a mutualistic association with fungi from the Glomeromycota Phylum forming the arbuscular mycorrhiza (AM) symbiosis. The establishment of this beneficial association requires an intensive signal exchange including the down-regulation of PAMP triggered responses. We have shown that secretion and delivery of the effector proteins contribute to the manipulation of the plant defense response. In a previous work we showed that one of this proteins travels to the plant nucleus and interacts with a plant transcription factor to down-regulate plant defenses. In order to identify further mechanisms of how symbiotic effectors function we are investigating new plant targets of mycorrhizal effector proteins and how do they differ from targets from pathogenic fungi. Progress in this area will be presented.

528. Structural basis for interactions of the Phytophthora sojae RXLR effector Avh5 with phosphatidylinositol 3-phosphate and for host cell entry. Furong Sun[2,3], Shiv Kale[4], Hugo Azurmendi[3], Dan Li[2], Brett M. Tyler[1,4], Daniel Capelluto[2]. 1) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 2) Dept of Biological Sciences, Virginia Tech, Blacksburg, VA 24061; 3) Dept of Chemistry, Virginia Tech, Blacksburg, VA 24061; 4) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061.

Oomycetes, such as Phytophthora sojae, employ protein effectors that enter host cells to facilitate infection. Entry of some effector proteins into plant cells is mediated by conserved RxLR motifs in the effectors and phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PtdIns(3)P). Recent reports differ regarding the regions on RxLR effector proteins involved in PIP recognition. To clarify these differences, we have structurally and functionally characterized the P. sojae effector, avirulence homolog-5 (Avh5). Using NMR spectroscopy, we demonstrate that Avh5 is helical in nature with a long N-terminal disordered region. Heteronuclear single quantum coherence titrations of Avh5 with the PtdIns(3)P head group, inositol 1,3-bisphosphate (Ins(1,3)P2), allowed us to identify a C-terminal lysine-rich helical region (helix 2) as the principal lipid-binding site in the protein, with the N-terminal RxLR (RFLR) motif playing a more minor role. Mutations in the RFLR motif slightly affected PtdIns(3)P binding, while mutations in the basic helix almost abolished it. Avh5 exhibited moderate affinity for PtdIns(3)P, which increased the thermal stability of the protein. Mutations in the RFLR motif or in the basic region of Avh5 both significantly reduced protein entry into plant and human cells. Both regions independently mediated cell entry via a PtdIns(3)P-dependent mechanism. Our findings support a model in which Avh5 transiently interacts with PtdIns(3)P by specific electrostatic interactions mainly through its positively charged helix 2 region, enabling the RFLR domain to promote PI3P-mediated host entry. This study, including the identification of the PtdIns(3)P-binding site, provides an improved and updated model for how RxLR effector proteins recognize phosphoinositides and for the contributions of the RxLR motif and basic-rich C-terminal regions to the internalization process.

529. Dose-dependent induction of plant immunity by application of the *Fusarium* mycotoxin deoxynivalenol. Antje Blümke, Christian A. Voigt. Molecular Phytopathology, Biocenter Klein Flottbek, Hamburg, Germany.

The mycotoxin deoxynivalenol (DON) is associated with Fusarium head blight (FHB). This disease causes vast losses by reducing grain quantity and quality. Our study was aimed at analyzing cell wall changes in the host model grass *Brachypodium distachyon* due to infection with different *F. graminearum* mutants. The mutants vary in their ability to infect spikelets and to produce DON. As a reference, we used a *F. graminearum* wild-type strain with the full capacity to produce DON and to cause FHB. The results were compared to the infection with the *Dtri5*, the *Dfgl1*, and the *Dgpmk1* mutant. The *Dtri5* mutant cannot produce DON due to disruption of the DON biosynthetic pathway. This mutant infects the directly inoculated spikelet without further propagation into the head tissue. A similar disease phenotype is described for the lipase-disruption mutant *Dfgl1*, which is able to produce DON. The MAP kinase disruption mutant *Dgpmk1* is apathogenic but still able to produce DON. We observed similar disease phenotypes and amounts of DON for all *F. graminearum* mutants during *B. distachyon* infection as described for wheat. 7 days post-inoculation (dpi), we analyzed the non-cellulosic monocarbohydrate cell wall composition of *B. distachyon* spikelets by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Only the infection with *F. graminearum* mutants that showed reduced virulence but still produce DON, namely the *Dfgl1* and *Dgpmk1* mutants, resulted in compositional changes of the cell wall, an increase in the amount of glucose. Next, we wanted to know to what extend the mycotoxin DON itself can induce cell wall changes. We applied DON solutions at different concentrations to *B. distachyon* spikelets. 7 dpi, the HPAEC-PAD analysis revealed an increase in the glucose amount only at relatively low DON concentrations of 1, 10, 100, and 1000 ppb whereas higher DON concentrations of 50 and 100 ppm did not change the cell wall composition. However, only these high DON concentrations caused necrosis of florets. Interestingly, *F. graminearum* wild-type infection was significantly reduced on spikelets sprayed with a DON solution at a concentration of 1000 ppb 7 days prior fungal inoculation. This suggests that the mycotoxin DON can induce an effector-triggered-like immunity in a dose-dependent manner.

530. How Oomycete Pathogens Exploit PI3P to Target Secreted RxLR Effectors into Host Cells. Q. Wang[1], S. Ferrer[1], J. Carlough[1], F. Arredondo[1], S. Kale[2], B. Tyler[1]. 1) Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR. 97330; 2) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24060.

Effector proteins from diverse oomycetes and fungi can enter plant cells to facilitate infection. Recent research suggests that phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PI3P) mediate the entry of some oomycete RxLR effectors. The PIP recognition domain of these effectors is still controversial. Current evidence shows that either the RxLR domain or positive residues in the C-terminal domains (Ct) of some effectors such as the P. sojae effectors Avr1b and Avh5 can bind PI3P, it has been unresolved which of these domains, if either, or both, are involved in cell entry during natural pathogen infection. Here we have used heterologous PI3P-binding proteins, such as the yeast VAM7p PX domain to replace the RxLR or Ct domains of Avr1b in P.sojae transformants. Our results reveal that the VAM7p PX domain can replace the RxLR domain of Avr1b in carrying the C-terminal domain of Avr1b into soybean cells, conferring an avirulent phenotype on the transformants. Mutations that abolish the binding of VAM7p to PI3P substantially reduce but do abolish avirulence conferred by the construct. Mutations in the PI3P-binding residues of the Avr1b Ct also substantially reduce avirulence, while the double mutant cannot confer avirulence at all. These results strongly support the hypothesis that PI3P-binding is essential for Avr1b cell entry during natural infection, and further suggest that efficient entry by Avr1b may require two PI3P binding sites.

531. Identification and functional assay of Phytophthora sojae avirulence effectors. Yuanchao Wang, Suomeng Dong, Weixiao Yin. Plant Pathology Dept, Nanjing Agri Univ, Nanjing, China.

Phytophthora sojae is a notorious oomycete pathogen producing a great loss on global soybean production annually. The disease outcome between soybean and P. sojae depends on whether hosts could recognize pathogen avirulence effectors. Recently identified oomycete avirulence effectors are characterized by N-terminal host entry motif (RxLR motif), sequence and transcriptional polymorphisms between virulent and avirulent strains. Benefit from 454 genome sequencing and solexa transcriptome sequencing of P. sojae strains, eight RxLR effectors are bioinformatically identified, genetic mapping suggested that two of them perfectly matched Avr3b and Avr1d phenotype respectively. Transient expression of the ORF from avirulence strain on soybean specifically triggered Rps3b and Rps1d mediated program cell death, respectively. confirming that they encodes avirulence effector Avr3b and Avr1d. Transient expression of Avr3b and Avr1d on Nicotiana benthamiana could promote the infection of Phytophthora capasici, suggesting both avirulence effectors could suppress plant immunity and contribute to pathogen infection. Silencing of Avr3b impaired the virulence of Phytophthora sojae. Our progress in elucidating the mechanism under the inhibiting plant immunity by these effectors will be presented.

532. Evaluating the translocation- and phospholipid binding abilities of the *Phytophthora infestans* AVR3a and *Phytophthora sojae* Avr1b RxLR-leaders. Stephan Wawra[1], Armin Djamei[2], Isabell Küfner[3], Thorsten Nürnberger[3], Justin A. Boddey[4], Stephen C. Whisson[5], Paul R.J. Birch[5], Regine Kahmann[2], Pieter van West[1]. 1) Sch Med Sci, Univ Aberdeen, Aberdeen, United Kingdom; 2) Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Germany; 3) Department of Plant Biochemistry, University Tübingen, Germany; 4) Department of Medical Biology, University of Melbourne, Australia; 5) Cell and Molecular Sciences, James Hutton Institute, Dundee, UK.

Plant pathogenic oomycetes have a large set of secreted effectors that are directed into their host cells during infection. One group of these effectors are the RxLR-effectors found in plant pathogenic oomycetes. These RxLR-effectors are defined as putative secreted proteins that contained a conserved tetrameric amino acid sequence motif, Arg-Xaa-Leu-Arg. This motif has to be within 40 amino acids C-terminal of the predicted cleavage sites of canonical signal peptides. Often this sequence is followed by a Glu-Glu-Arg (EER) motif. It has been shown, in a few cases, that the RxLR-motif is important for the delivery of these proteins into host cells. However, how these proteins translocate into the cytoplasm of their host is currently the object of intense research activity and debate. One model suggests that the RxLR-leader sequences of these effectors are sufficient to translocate the respective effectors into eukaryotic cells through binding to surface exposed phosphoinositol-3-phosphate. However, analysing the translocation behaviour of the RxLR-leaders from *Phytophthora infestans* avirulence protein 3a (AVR3a) and *Phytophthora sojae* avirulence protein 1b (Avr1b) we were unable to obtain conclusive evidence for specific RxLR-mediated translocation. Importantly, we confirm that the reported phospholipid binding properties of AVR3a and Avr1b are not mediated by their RxLR-leaders. In addition, we will present data showing that the observed phospholipid interaction of the AVR3a effector domain is attributable to a weak association with denatured protein molecules, and is therefore most likely physiologically irrelevant.

533. Identifying essential effectors from the soybean pathogen *Phytophthora sojae*. Hua Z. Wise[1,2], Ryan G. Anderson[3], John M. McDowell[3], Brett M. Tyler[1,2]. 1) Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University; 2) Virginia Bioinformatics Institute, Virginia Tech; 3) Department of Plant Pathology, Physiology and Weed Science, Virginia Tech.

Breeding for resistance to plant pathogens is one of the most effective means of disease control. However, the ability of plant pathogens evolve new pathogenicity factors and evade host defense mechanisms drives the continual necessity to identify new resistance genes. We are exploiting genomic technologies in an effector-directed breeding approach that augments traditional breeding efforts against *Phytophthora sojae*, the causal agent of soybean root and seedling rot. This approach is founded on identifying monomorphic *P. sojae* effector genes that are essential for virulence, and using these genes as probes to identify new sources of resistance in soybean and related legumes. These essential effectors will make excellent candidates for screening for new, durable resistance to *P. sojae*, as these genes presumably cannot be mutated or deleted without a significant fitness penalty. The majority of predicted *P. sojae* RXLR effector genes are polymorphic amongst sequenced isolates of *P. sojae*, however, a subset of *P. sojae* RXLR effectors displays little or no allelic diversity. We have established a workflow for transient gene silencing and quantitative virulence assays. To date, we have silenced and assessed the virulence contribution of 17 *PsAvh* genes. Silencing of 13 of these effectors produced reduced virulence. Among these effectors, Avh16, Avh180 and Avh240 showed substantially reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. We are currently using these three effectors as candidates in a high throughput screen system utilizing *Pseudomonas* Type III secretion system to screen for new resistance genes against *P. sojae*.

534. The LysM effector, Ecp6, is a virulence factor in the interaction of the hemibiotroph, Setosphaeria turcica, but not the necrotroph, Cochliobolus heterostrophus, with their common host, maize. Dongliang Wu[1], Qing Bi[2], Gillian Turgeon[1]. 1) Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA; 2) State Key Program of Microbiology and Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, China, 300071.

Fungal phytopathogens are characterized as biotrophs, which derive nutrients from living cells, and necrotrophs, which kill host cells and retrieve nutrients from dead tissue. Hemibiotrophs are intermediate in that they initially establish themselves in living host tissue, then undergo rapid killing of plant cells later on. Hemi- and bio-trophic fungi utilize specialized effectors to prevent host recognition triggered by pathogen associated molecular patterns (PAMPs), whereas necrotrophs often produce toxins that induce programmed cell death. Chitin, a signature component of fungal cell walls, is one type of PAMPs known to trigger the plant resistance response. Several fungal chitin-binding LysM effectors have been identified that broker counter-defense against chitin-triggered immunity, including the first characterized one, Ecp6, from the tomato biotroph, Cladosporium fulvum, Mg3LysM, from the wheat hemibiotroph, Mycosphaerella graminicola, and Slp1, from the rice hemibiotroph, Magnaporthe oryzae. In this study, Ecp6 homologs were identified and deleted from the genomes of two maize pathogens which differ in pathogenic lifestyle, Setosphaeria turcica (hemibiotroph), causal agent of Northern Leaf Blight and Cochliobolus heterostrophus (necrotroph), causal agent of Southern Corn Leaf Blight. Deletion of StECP6 caused reduced virulence, whereas absence of ChECP6 did not alter virulence to the host. Real time RT-PCR demonstrated that expression of pathogenesis related maize genes, PR1 gene and a chitinase gene was increased in Stecp6 mutants compared to wild type at 4 days post inoculation. Additional in planta gene expression analyses are underway to compare host responses to these two fungi differing in pathogenic lifestyle on the same host.

535. Nematode-trapping fungi eavesdrop on nematode pheromones. Yen-Ping Hsueh[1], Parag Mahanti[2], Frank Schroeder[2], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Inst of Technology, Pasadena, CA; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

The recognition of molecular patterns associated with specific pathogens or food sources is fundamental to ecology and plays a major role in the evolution of predator-prey relationships. Recent studies showed that nematodes produce an evolutionarily highly conserved family of small molecules, the

ascarosides, which serve essential functions in regulating nematode development and behavior. Here we show that nematophagous fungi, natural predators of soil-dwelling nematodes, can detect and respond to ascarosides. Nematophagous fungi use specialized trapping devices to catch and consume nematodes, and previous studies demonstrated that most fungal species do not produce traps constitutively but rather initiate trap-formation in response to their prey. We found that ascarosides, which are constitutively secreted by many species of soil-dwelling nematodes, represent a conserved molecular pattern used by nematophagous fungi to detect prey and trigger trap formation. Ascaroside-induced morphogenesis is conserved in several closely related species of nematophagous fungi and occurs only under nutrient-deprived condition. Our results demonstrate that microbial predators eavesdrop on chemical communication among their metazoan prey to regulate morphogenesis, providing a striking example of predator-prey co-evolution. We anticipate that these findings will have broader implications for understanding other inter-kingdom interactions involving nematodes, which are found in almost any ecological niche on Earth.

536. Molecular diagnosis to discriminate pathogen and apathogen species of the hybrid *Verticillium longisporum* on the oilseed crop *Brassica napus*. Van Tuan Tran, Susanna Braus-Stromeyer, Christian Timpner, Gerhard Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany.

The cruciferous fungal pathogen *Verticillium longisporum* represents an allodiploid hybrid with long spores and almost double the amount of nuclear DNA compared to other *Verticillium* species. *V. longisporum* evolved at least three times by hybridization. In Europe, virulent A1xD1 and avirulent A1xD3 hybrids were isolated from the oilseed crop *Brassica napus*. Parental A1 or D1 species are yet unknown whereas the D3 lineage represents *Verticillium dahliae*. The *V. longisporum* isolates from Europe or California corresponding to hybrids A1xD1 or A1xD3 were analyzed. Only one single characteristic type of ribosomal DNA (rDNA) could be assigned to each hybrid lineage. The avirulent A1xD3 isolates carried exclusively D3 rDNA, which corresponds to *V. dahliae*, whereas the rDNA of the virulent A1xD1 isolates originates from A1. Both hybrid lineages carry distinct isogene pairs of conserved regulatory genes corresponding to either A1 or D1/D3. D1 and D3 paralogues show high identities but differ in several single nucleotide polymorphisms. Distinct signatures of the *VTA2* regulatory isogene pair allow the identification of *V. longisporum* hybrids by a single PCR and the separation from haploid species as A1 or D1/D3. The combination between the *VTA2* marker as a barcode marker and differentiation of the rDNA type represents an attractive diagnostic tool to discriminate allodiploid from haploid *Verticillia* and to distinguish between A1xD1 and A1xD3 hybrids, which differ in their virulence towards *B. napus*. Furthermore, the *VTA2* gene was demonstrated to be a virulence factor that is required for fungal morphogenesis and plant infection.

537. Investigating the Pathogenicity of *Armillaria*. Kathryn Ford[1], Beatrice Henricot[3], Kendra Baumgartner[2], Gary D. Foster[1], Andy M. Bailey[1]. 1) Molecular Plant Pathology, University of Bristol, Bristol, United Kingdom; 2) USDA-ARS, Plant Pathology, University of California, Davis, CA; 3) Royal Horticultural Society, Plant Pathology, Surrey, United Kingdom.

Armillaria sp., or 'honey mushroom', is a generalist pathogen of fruit, nut and timber trees in gardens, forests and agricultural systems worldwide, causing Armillaria root disease and resulting in significant yield losses and millions of dollars worth of damage annually. Several questions regarding the infection mechanisms used by basidiospores, hyphae and rhizomorphs and their subsequent colonisation processes remain unanswered. We established a reproducible method of producing fruiting bodies in culture in order to generate basidiospores for use in *Agrobacterium*-mediated transformation to facilitate further exploration of *Armillaria*'s pathogenicity. Results will be presented on the construction and utilisation of various plasmids conferring hygromycin resistance and fluorescent protein expression that have been used to transform *A. mellea* in order to study the infection mechanisms in herbaceous plants.

538. Detoxification of nitric oxide by flavohemoglobin and the denitrification pathway in the maize pathogen *Fusarium verticillioides*. Thomas Baldwin[1,2], Anthony Glenn[2]. 1) Plant Pathology Department, Univ of Georgia, Athens, GA; 2) USDA, ARS, R.B. Russell Research Center, Toxicology and Mycotoxin Research Unit, , Athens, GA.

The ephemeral nitric oxide (NO) is a free radical, highly reactive, environmentally rare, and a potent signaling molecule in organisms across kingdoms of life. This gaseous small molecule can freely transverse membranes and has been implicated in aspects of pathogenicity both in animal and plant hosts. *Fusarium verticillioides* is a mycotoxigenic pathogen of maize, notable for its ability to persist as an asymptomatic endophyte. One potential determinant of this lifestyle conversion between overt pathogen and symptomless endophyte may be the regulation of NO. Detoxification of NO is a known pathogenicity factor for the fungal human pathogen *Candida albicans* and the bacterial plant pathogen *Erwinia chrysanthemi*. Both mediate detoxification by a flavohemoglobin protein (CaYHB1 and HmpX, respectively). BLASTP search of the *F. verticillioides* genome revealed two putative flavohemoglobin homologs, denoted FHB1 and FHB2. Microarray analysis revealed a significant induction of *FHB2* (13-fold) when the fungus was exposed to exogenous NO. *FHB1* had a 2-fold increase. Also noteworthy from the microarray data is the distinct induction of genes within the denitrification pathway, including dissimilatory nitrate reductase (dNaR, 16-fold increase), dissimilatory nitrite reductase (dNiR, 226-fold), and P450 nitric oxide reductase (P450nor, 27-fold). Flavohemoglobin has been noted as a component of the denitrification pathway, having a role in converting NO to nitrate. Thus, *FHB2* is postulated to be the paralog involved in the *F. verticillioides* denitrification pathway. Deletion mutants are being created in dNiR, P450nor, *FHB1*, and *FHB2* to further evaluate functions of these genes in *F. verticillioides*. Mutants will be assayed for their endogenous production and regulation of NO, response to exogenous NO, virulence against maize, and mycotoxin production. Elucidating the function of these genes will give insight into the role of NO in *F. verticillioides* development, maize-fungal interactions, and denitrification, which has previously only been assessed in relation to anaerobic growth.

539. Family disintegration: One *Fusarium verticillioides* beta-lactamase gene at a time. Scott E. Gold, Xiu Lin, Nicole J. Crenshaw, Anthony E. Glenn. Toxicology & Mycotoxin Research, USDA-ARS, Athens, GA.

Fusarium verticillioides is a mycotoxigenic fungus found commonly on maize, where it primarily exhibits asymptomatic endophytic growth. The *F. verticillioides* genome possesses approximately 30 regions that potentially encode beta-lactamase enzymatic domains. These enzymes are classically involved in bacterial resistance to beta-lactam antibiotics, for example penicillinase. Our attention was drawn to this enzymatic function by the recent finding that the gene FVEG_08291 is essential for resistance to maize phytoanticipins such as 2-benzoxazolinone (BOA), which possesses a gamma-lactam moiety, the presumed enzymatic target (see poster by Glenn et al.). FVEG_08291 belongs to a subset of these enzymes known as metallo-beta-lactamases. Beta-lactamase enzyme function is not well studied in the fungi, so, in order to further evaluate the roles of these enzymes in *F. verticillioides*, we are in the process of deleting the members of their encoding gene family. We assigned directed-research undergraduates each a specific gene, for which they produced deletion constructs by DelsGate and/or OSCAR methodology and generated fungal transformants for analysis. Deletion mutants in one of the other metallo-beta-lactamase encoding genes (FVEG_12159) showed a dramatic defective growth phenotype. This observation raises the interesting hypothesis that perhaps this mutant is no longer resistant to a lactam moiety containing compound produced by *F. verticillioides* itself. Data will be presented on initial progress with this project.

540. A Fungal Metallo-Beta-Lactamase Necessary for Biotransformation of Maize Phytoprotectant Compounds. Anthony E. Glenn, C. Britton Davis, Maurice E. Snook, Scott E. Gold. Toxicology & Mycotoxin Research, USDA-ARS, Athens, GA.

Xenobiotic compounds such as phytochemicals, microbial metabolites, and agrochemicals can impact the diversity and frequency of fungal species occurring in agricultural environments. Resistance to xenobiotics may allow plant pathogenic fungi to dominate the overall fungal community, with potential negative impacts on crop yield and value. The mycotoxigenic *Fusarium verticillioides* is such a fungus commonly associated with maize worldwide, often contaminating maize kernels with the fumonisin mycotoxins. The dominance of *F. verticillioides* as an endophyte may be due in part to its ability to metabolize phytoprotectants produced by maize. The benzoxazinoids and benzoxazolinones are broad spectrum allelopathic, antimicrobial, and anti-herbivory compounds from maize, yet *F. verticillioides* can rapidly biotransform these phytochemicals into non-toxic metabolites. We have identified the genes responsible for the biotransformation process. Two gene clusters were identified that correspond to the previously characterized FDB1 and FDB2 loci, with both loci being necessary for metabolic tolerance to 2-benzoxazolinone (BOA), one of the maize phytoprotectants. Analysis of the nine ORFs (FVEG_08287 to FVEG_08295) at the FDB1 locus indicated that one of the genes (FVEG_08291) encodes a protein having a metallo-beta-lactamase domain, and deletion of the gene in wild-type strain M3125 resulted in the fungus being unable to grow on BOA-amended agar due to an inability to metabolize the compound. Deletion mutants were complemented to wild-type phenotype by transformation with the native allele. Other ORFs were not found to be essential when deleted in M3125. Microarray analysis indicated the metallo-beta-lactamase (FVEG_08291) had a 13-fold induction in response to BOA (2-hr incubation), with other genes in the cluster ranging from 3-fold (FVEG_08287) to 42-fold induction (FVEG_08292). Beta-lactamases are well-known for conferring bacterial resistance to lactam-type antibiotics, but to our knowledge this is the first report of fungal enzymes of this type metabolizing lactam-like xenobiotics. We are investigating other beta-lactamase encoding genes in *F. verticillioides* (see poster by Gold et al.) to further evaluate their possible role in tolerance to both exogenous as well as endogenous metabolites having lactam-type moieties.

541. *Nectria haematococca* DNase: role in dynamics and localization of pea root infection. D. Huskey, G. Curlango-Rivera, Z. Xiong, H. Van Etten, M. Hawes. University of Arizona, Tucson, AZ.

Root tips of pea (*Pisum sativum* L.) are protected from *N. haematococca* infection by an extracellular DNA (exDNA)-based trapping process similar to that occurring in mammalian defense responses to bacterial and fungal pathogens. *N. haematococca* spores germinate rapidly in response to root border cell populations programmed to export exDNA and antimicrobial proteins as they detach from the root cap. Within 24 h, hyphae and border cells together form a mantle which ensheaths the root tip and separates, leaving the root apex uninvaded: >98% of root tips escape infection. When the exDNA is degraded with DNase added to the root at the time of inoculation, resistance is abolished: 100% of root tips are invaded by proliferating hyphae and root growth ceases within 24 hours. In *N. haematococca* isolates harboring a conditionally dispensable (CD) chromosome, the process occurs more rapidly than in CD-minus isolates; a ten-fold increase in spores from CD-minus isolates results in comparable dynamics. Putative DNase-encoding sequences have been detected on two different CD chromosomes, and direct tests have revealed increased extracellular DNase activity from CD-plus isolates compared with CD-minus isolates. The goal of this study is to examine predictions of the hypothesis that CD chromosome encoded DNase activity plays a role in pea root infection.

542. *FvSNF1*, a protein kinase of *Fusarium virguliforme* that affects SDS development in Soybean. K.T. Islam, Ahmad Fakhoury. Plant, Soil and Ag Systems, Southern Illinois University, Carbondale, IL.

Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS). SDS is one of the top four yield-robbing fungal diseases in soybean resulting in significant economic losses to producers. Despite the importance of SDS, a clear understanding of fungal genetic factors that affect the development of the disease is still lacking. The aggressiveness of *F. virguliforme* on infected soybean plants is believed to require the activity of cell wall-degrading enzymes (CWDE). The production of these CWDEs in phytopathogenic fungi is under catabolic repression. In *Saccharomyces cerevisiae*, catabolic repression is regulated by SNF1 (sucrose non-fermenting 1). To investigate the role of cell wall-degrading enzymes as determinants of *F. virguliforme* aggressiveness, the *F. virguliforme* SNF1 homologue *FvSNF1* was targeted for disruption. The resulting *FvDsnf1* transformant failed to grow on galactose and grew poorly when arabinose or sucrose where the main carbon source. The mutation did not seem to affect the ability of the fungus to grow with glucose, fructose, maltose, or xylose as the main source of carbon. More importantly, in greenhouse experiments, the *FvDsnf1* transformant was severely impaired in its ability to cause SDS on challenged soybean plants.

543. Functional and molecular analysis of AstA sulfate transporter in pathogenic *Fusarium sambucinum* with respect to its virulence and ability to infect potato. Sebastian Pilsyk[1], Hanna Gawinska-Urbanowicz[2], Renata Natorff[3], Marzena Sienko[3], Joanna S. Kruszewska[1]. 1) Laboratory of Fungal Glycobiology, Institute of Biochemistry and Biophysics, Warsaw, Poland; 2) The Plant Breeding and Acclimatization Institute (IHAR), Bonin, Poland; 3) Department of Genetics, Institute of Biochemistry and Biophysics, Warsaw, Poland.

AstA protein (alternative sulfate transporter) represents a little known type of sulfate trasporter, belonging to an extensive and poorly characterized family of allantoate permeases Dal5. In *Aspergillus nidulans* the *astA* gene is under the control of Sulfur Metabolite Repression (SMR). The closest homologs of *astA* are frequent in evolutionarily distant fungi belonging to the *Pezizomycotina* subphylum which exhibit similar plant pathogenicity. *Fusarium* sp. fungi, like *F. sambucinum*, contribute to serious devastation of potato crops and increase the cost of cultivation due to the application of pesticides. Due to the similarity on the metabolic level between pathogenic fungi and the host, there is a problem with efficient plant protection.

The aim of this project is elucidation of AstA function upon infection and colonization of potato tubers by the fungal pathogen, *Fusarium sambucinum* and by its *astA* deletion mutant. We have observed a high expression level of *astA* in infected potato tubers and its regulation by SMR as in *A. nidulans*.

The study also involves the identification of amino acid residues crucial for sulfate binding and transportation by the generation of point mutations and uptake analysis. Elucidation of the biological function of AstA will help understanding of fungal pathogenic adaptations upon changes in plant host metabolism and definition of a new promising target for a potential fungicide.

544. WITHDRAWN

545. The adenylate cyclase of the cereal pathogen *Fusarium graminearum* controls infection structure development, mycotoxin production and virulence to wheat. Jörg Bormann, Marike Johanne Boenisch, Elena Brückner, Demet Firat, Cathrin Kröger, Birgit Hadeler, Wilhelm Schäfer. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

Fusarium graminearum is one of the most devastating pathogens of cereals. Mycotoxins accumulating in infected grains are a serious threat to food and feed worldwide. Knowledge about the molecular basis of infection and mycotoxin production is still limited. Cyclic 3',5'-adenosine monophosphate (cAMP)

is a nucleotide derived from adenosine triphosphate that acts as a second messenger throughout all kingdoms. Intracellular cAMP levels are subject to a large membrane-bound protein, the adenylate cyclase. In order to analyze the function of this gene and the importance of cAMP in the life cycle of *F. graminearum*, the adenylate cyclase gene (FGSG_01234) was deleted from the genome (D*Fgac1*). D*Fgac1* displayed a drastically reduced growth on complete medium. This reduction in growth could partially be complemented by addition of a cAMP analog. Furthermore, the mutant was unable to produce perithecia on detached wheat nodes but more artificial conditions like carrot agar allowed perithecia development. Possibly, this points to a sensing problem of D*Fgac1*. Although growth on agar was reduced, conidia production was increased. Pathogenicity towards wheat was drastically reduced in D*Fgac1* compared to the wild type. Point-inoculated spikelets showed only small lesions even after 21 days post inoculation. No deeper infection occurred and mycelial growth was never detectable near the rachis. Thus, fungal hyphae never grew from the inoculated spikelet to the adjacent one. Fluorescence microscopy using a D*Fgac1*-strain expressing dsRed constitutively in the cytosol revealed that FgAC1 controls the development of infection structures like lobate appressoria and infection cushions. Removal of hyphae superficially colonizing flower leaves and subsequent analysis by scanning electron microscopy demonstrated the lack of any fungal penetration holes. Instead, hyphae on flower leaves produced massively new conidia, thereby circumventing the infection cycle, something never observed in the wild type. D*Fgac1*-strains are unable to produce the mycotoxin deoxynivalenol both in vitro and during wheat infection. In this study, for the first time, we implicate the cAMP signaling pathway to important processes in *F. graminearum* like development of infection structures, pathogenicity, secondary metabolite production and sexual reproduction.

546. The ATF/CREB transcription factor Atf1 is essential for full virulence, deoxynivalenol production and stress tolerance in the plant pathogen *Fusarium graminearum*. Thuat Van Nguyen, Birgit Hadeler, Cathrin Kröger, Wilhelm Schäfer, Jörg Bormann. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

The filamentous ascomycete *Fusarium graminearum* is a highly organ specific pathogen that resides on small grain cereals like rice, wheat, barley, and maize. Grains infected with *F. graminearum* accumulate high amounts of mycotoxins, most prominent of which are deoxynivalenol (DON) and zearalenone (ZEA). The stress-activated MAP-kinase FgOS-2 (*Saccharomyces cerevisiae* HOG1) is a central regulator in the life cycle of *F. graminearum* (Nguyen et al., 2012. MPMI 25:1142-1156). FgOS-2 regulates, among others, virulence to wheat and maize, and DON- and ZEA-production. Here, we present data on the functional characterization of a putative downstream regulator, the ATF/CREB activating transcription factor FgAtf1. We created deletion and over-expression mutants of *Fgatf1*, the latter one also in an *FgOS-2* deletion mutant. Like FgOS-2, FgAtf1 is mainly involved in osmotic stress response. Bimolecular fluorescence complementation demonstrates an interaction of both proteins under osmotic stress conditions. Deletion mutants in *Fgatf1* (D*Fgatf1*) are more sensitive to osmotic stress (e.g. mediated by NaCl) and less sensitive to oxidative stress mediated by H_2O_2 compared to the wild type. Furthermore, sexual reproduction is delayed: perithecia develop much slower and some remain immature even after prolonged incubation. D*Fgatf1* strains show an increased DON-production under in-vitro induction conditions compared to the wild type. However, during wheat infection, DON-production is strongly reduced. Expression of genes encoding for key enzymes in the DON-biosynthesis pathway is regulated accordingly. In infection assays on wheat and maize, the D*Fgatf1* strains show a reduced virulence compared to the wild type. Interestingly, constitutive expression of *Fgatf1* leads to hypervirulence on wheat, maize and *Brachypodium distachyon*. Moreover, constitutive expression of *Fgatf1* in a D*FgOS-2* mutant background partially complements D*FgOS-2*-phenotypes regarding growth on osmotic-stress medium, sexual reproduction, and virulence towards wheat and maize. Furthermore, FgAtf1 is involved in the regulation of light-responsive genes. Taken together, these results provide new insights in the stress response signaling cascades of *F. graminearum* and assign the transcription factor FgAtf1 a central role in pathogenic development and secondary metabolism.

547. The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*. Thuat Van Nguyen, Birgit Hadeler, Cathrin Kröger, Wilhelm Schäfer, Jörg Bormann. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

Fusarium graminearum is one of the most destructive pathogens of cereals and a threat to food and feed production worldwide. It is an ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease in maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Zearalenone (ZEA) and deoxynivalenol (DON) are hazardous mycotoxins; the latter is necessary for virulence towards wheat. Deletion mutants of the *F. graminearum* orthologue of the *Saccharomyces cerevisiae* Hog1 stress-activated protein kinase, FgOS-2 (D*FgOS-2*), showed drastically reduced in planta DON and ZEA production. However, D*FgOS-2* produce even more DON than the wild type under in vitro conditions, whereas ZEA production is similar to that of the wild type. These deletion strains are dramatically reduced in pathogenicity towards maize and wheat. We constitutively expressed the fluorescent protein dsRed in the deletion strains and the wild type. Microscopic analysis revealed that D*FgOS-2* is unable to reach the rachis node at the base of wheat spikelets. During vegetative growth, D*FgOS-2* strains exhibit increased resistance against the phenylpyrrole fludioxonil. Growth of mutant colonies on agar plates supplemented with NaCl is reduced but conidia formation remained unchanged. However, germination of mutant conidia on osmotic media is severely impaired. Germ tubes are swollen and contain multiple nuclei. The deletion mutants completely fail to produce perithecia and ascospores. Furthermore, FgOS-2 also plays a role in reactive oxygen species (ROS)-related signalling: *FgOS-2* deletion mutants are more resistant against oxidative stress mediated by H_2O_2. We found that the transcription and activity of fungal catalases is modulated by FgOS-2. Among the genes regulated by FgOS-2 we found a putative calcium-dependent NADPH-oxidase (*noxC*) and the transcriptional regulator of ROS metabolism, *Fgatf1*. The present study describes new aspects of stress-activated protein kinase signalling in *F. graminearum*.

548. Innate Immunity in *Fusarium graminearum*. Vong shian Simon Ip Cho[1,2], Gitte Erbs[3], Thomas Sundelin[3], Peter Busk[4], Mari-Anne Newman[3], Stefan Olsson[1]. 1) Genetics and Microbiology, University of Copenhagen, Copenhagen, Denmark; 2) USDA-ARS Cereal Disease Laboratory, University of Minnesota, Saint Paul, MN, USA; 3) Transport Biology, University of Copenhagen, Copenhagen, Denmark; 4) Dept. Biotechnology, Aalborg University, Copenhagen, Denmark.

Fungi are often mostly recognized as plant pathogens that cause harm to important economical plants. In nature however, fungi are frequently victims of bacterial parasitism but little is known about fungal defense mechanisms. The potential existence of fungal innate immunity was studied using *Fusarium graminearum* as model organism and bacterial flagellin to mimic the presence of bacteria in an *in vitro* environment. The presence of flagellin triggered an initial mitochondrial and cell membrane hyperpolarization which was detected using the florescent dye $DiOC_7(3)$. This was followed by the production of the secondary signalling molecule Nitric Oxide (NO), common to innate immunity signalling in other eukaryotes. NO was monitored using the fluorescent dye DAF-FM. NO appears to be produced by an inducible enzyme that is regulated by complex mechanisms but centrally modulated by Calcium/Calmodulin. Inhibition studies suggest the presence of a Nitric Oxide Synthase (NOS), but no typical arginine utilizing NOS was identified within the *F. graminearum*'s genome by homology search. Various genes bearing resemblance to the archetypal NOS, as well as argininosuccinate lyase were deleted. However, the mutants still produced NO. The presence of alternative pathways contributing towards the production of NO was investigated by adding a variety of potential substrates to challenged cultures. Various reactions were observed suggesting that several pathways are present. In conclusion, *F. graminearum* reacts strongly to the presence of the bacterial Microbial Associated Molecular Pattern (MAMP) flagellin with an up-regulation

of NO production showing the presence of innate immunity-like responses also in fungi.

549. Balanced posttranslational activation of eukaryotic translation initiation factor 5A is required for pathogenesis in *Fusarium graminearum*. Ana Lilia Martinez-Rocha[1], Mayada Woriedh[1,2], Jan Chemnitz[3], Peter Willingmann[1], Joachim Hauber[3], Wilhelm Schäfer[1]. 1) Molecular Phytopathology, University of Hamburg, Hamburg, Hamburg, Germany; 2) Cell Biology and Plant Biochemistry, University of Regensburg, Germany; 3) 3Heinrich-Pette-Institute for Experimental Virology and Immunology, Martinistrasse 52, D- 20251 Hamburg, Germany.

Activation of the eukaryotic translation initiation factor 5A (EIF5A) requires a posttranslational modification, changing a lysine into the unique amino acid hypusine. This activation is a two steps reaction mediated by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). First DHS cleavage and transfers the 4-aminobutyl moiety from the spermidine to an specific lysine in EIF5A to form the deoxyhypusine intermediate, during the second step DOHH hydroxylate the 4-amonibutyl moiety to create the active form of eIF5A containing hypusine. The activated protein transports mRNAs from the nucleus to the ribosomes, where initiates protein biosynthesis. This system is conserved from Archea to humans and is involved in diseases as diverse as HIV infection, malaria, cancer, and diabetes. Until now, inhibition or silencing of DHS or DOHH has been tested to modify hypusination. For the first time, we evaluate its importance by over-expressing the enzymes that control hypusination of EIF5A. Over-expression of DOHH (oexDOHH) prevents virulence of *Fusarium graminearum* to wheat and maize. In contrast, over-expression of DHS (oexDHS) leads to an increase of virulence to wheat. Simultaneous over-expression of both enzymes results in virulence comparable to the wild type strain. GFP assisted histology revealed that oexDOHH mutant is unable to form infection structures on wheat flower leaves and barely grows in point inoculated wheat spikelets. OexDHS results in an increase of infection structures and accordingly in an increase of virulence. We determined general hypusine formation by incorporating radiolabeled spermidine in EIF5A during in culture growth. Wild type and oexDHS showed similar hypusination intensity, whereas oexDOHH showed an increased incorporation. The differential hypusination state of EIF5A was determined by 2D gels and western blot due to the difference in isoelectric point of the three states; inactive (lysine), intermediate (deoxyhypusine) or active (hypusine). Preliminary results show the wild type strain with all three hypusination states, oexDHS and double over-expressing mutants with an increased inactive and intermediate forms, and oexDOHH mutant lacked the inactive and intermediate form, only the activated form was detectable. We conclude that a balanced hypusination is required for proper function of EIF5A.

550. The Con7 transcription factor, essential for pathogenicity, regulates the expression of genes involved in glycolysis and virulence in *Fusarium oxysporum*. Carmen Ruiz-Roldán[1,2], Yolanda Pareja-Jaime[1,2], M. Isabel G. Roncero[1,2]. 1) Department of Genetics, University of Cordoba, Spain; 2) Campus de Excelencia Agroalimentario (ceiA3).

Transcription factors (TF) regulating the different stages of infection like adhesion to the host surface, differentiation of infection structures and penetration represent potential molecular targets for fungicides with specific modes of action. Our studies on *Fusarium oxysporum* have demonstrated the essential role of morphogenetic regulation in pathogenesis, including processes such as cell-wall biogenesis, cell division and differentiation of infection structures. We identified the Con7 TF whose inactivation produces non-pathogenic mutants with altered morphogenesis, including abnormal polar growth and hyphal branching. To identify genes dependent on Con7 protein profiles of wild type and D*con7* mutant were compared by 2D electrophoresis. Expression of 126 proteins varied quantitatively between both strains with statistical significance. Among the 80 proteins identified by MALDI-TOF/TOF-MS 15, 9, and 4 were associated with secondary metabolism, glycolysis/gluconeogenesis (Gly/Glu) and pentose and glucuronate interconversions, respectively. Additionally, 6 proteins were known virulence factors, including citochrome P450 monooxygenase, O-methyltransferase, peptidyl-prolyl cis-trans isomerase D, peroxidase/catalase, phospholipase C and superoxide dismutase. Expression of the responsible genes was confirmed by qRT-PCR. To verify the role of Con7 in Gly/Glu pathways, the intracellular ATP levels and alcohol dehydrogenase (ADH) activity in D*con7* were compared with wt. We detected 2.4 fold increased ATP and 27.5% reduced ADH activity in D*con7*. Additionally, D*con7* showed a dramatic growth reduction in the presence of glucose, glycin or polygalacturonic acid, indicating nutrient assimilation defects. No significant differences were detected in intracellular glucose accumulated by both strains, while extracellular glucose levels in D*con7* were significantly higher, suggesting defective sugar transport. RT-PCR analyses in the wt revealed the existence of four *con7* transcripts that differ in size and abundance, indicating alternative intron splicing. To determine the functionality of the different deduced proteins, D*con7* was complemented with cDNA fragments from each version of the mature Con7 protein. Phenotypic and pathotypic characterization of the transformants should reveal their role in the different phenotypes observed in the D*con7*.

551. *Fusarium oxysporum* produces volatile organic compounds that enhance the growth and stress resistance of *Arabidopsis thaliana*. Vasileios Bitas[1], Michael Axtell[2], James Tumlinson[3], Seogchan Kang[1]. 1) Department Plant Pathology and Environmental Microbiology, Pennsylvania State Univ, University Park, PA; 2) Department of Biology, Pennsylvania State Univ, University Park, PA; 3) Department of Entomology, The Pennsylvania State University, University Park, PA.

Production of volatile organic compounds (VOCs) as signal molecules is a widespread and efficient mode of communication utilized by all organisms. Microbial VOCs promoting plant growth and stress resistance present an environmentally and economically attractive alternative to fertilizers and pesticides. Fungi are some of the most predominant and yet, under-investigated organisms that employ VOCs in order to regulate and affect surrounding environments including neighboring organisms. Certain isolates of *F. oxysporum*, a well-known soilborne fungus, produce VOCs that promote plant growth, alter morphological and physiological properties, and enhance biotic and abiotic stress resistance in the model plant A*rabidopis thaliana*. In order to investigate the mode of action by which those volatiles function, we have employed genetic and molecular resources and tools including *A. thaliana* mutants that are defective hormonal signaling pathways and gene expression analysis. We are also trying to identify those compounds that affect *A. thaliana* through the use of GC-MS. Identification of the fungal VOCs triggering these plant responses and elucidation of the physiological and molecular alterations occurring in plants will help us shed light into the mechanism underpinning complex VOC-mediated interactions.

552. Lipolytic system of the tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici*. G. A. Bravo Ruiz[1,2], C. Ruiz Roldán[1,2], M. I. González Roncero[1,2]. 1) Departamento de Genética, Universidad de Córdoba; 2) Campus de Excelencia Agroalimentario (ceiA3), E-14071 Cordoba, Spain.

The lipolytic profile of *Fusarium oxysporum* f.sp *lycopersici* has been determined on the basis of in silico analyses search and validated by biochemical enzyme activity determination of Wheat Germ Oil (WGO) induced cultures. Twenty five identified structural secreted lipases show the conserved pentapeptide -Gly-X-Ser-X-Gly- characteristic of fungal lipases and the signal sequence for extracellular secretion. On the other hand, two transcriptional regulatory predicted lipase genes have been identified showing nuclear localization signals (NLS) and the Zn2Cys6 zinc finger DNA binding motifs. The transcription profile of twelve structural genes, during tomato plant colonization in the wild type strain, shows variable expression levels (100 fold-range) being *lip1*, *lip3*, and *lip22* the highest induced (20% relative to the actin gene). The maximal level of expression is observed in roots at 21-96 hours post-inoculation. Targeted replacement of four structural (*lip1*, *lip2*, *lip3* and *lip5*), and two regulatory *ctf1* (orthologue to *Fusarium solani ctf1* and to *Aspergillus nidulans farA*) and *ctf2* (orthologue to *F. solani ctf2* and to *A. nidulans farB*), lipase predicted genes originated the corresponding single deletion mutants

and the double deletion mutant D*ctf1*D*ctf2*. In vitro qRT-PCR expression analyses of twelve structural lipase genes in the regulatory mutants D*ctf1*, D*ctf2* and D*ctf1*D*ctf2*, in comparison with the wild type strain, demonstrate the existence of a complex lipase regulation network in *F. oxysporum*. The reduction of total lipase activity (10-30%), besides the severe altered pathogenic behaviour on tomato plants shown by the single D*ctf1*, D*ctf2*, and the double D*ctf1*D*ctf2* mutants suggest an important role of the lipolytic system of this fungus in pathogenicity.

553. Role of glycogen metabolism in the pathotypic behavior of *Fusarium oxysporum* f.sp. *lycopersici* on tomato plants. C. Corral Ramos[1,2], C. Ruiz Roldan[1,2], M. I. González Roncero[1,2]. 1) Departamento de Genética, Universidad de Córdoba; 2) Campus de Excelencia Agroalimentario (ceiA3), E-14071 Córdoba, Spain.

Glycogen can play different roles in biological systems, such as storage carbohydrate for energy and/or carbon or control of glucose metabolism. In most eukaryotes, glycogen is built on a self-glucosylating initiator protein core, glycogenin (*gnn*), which acts as a primer for glycogen synthase (*gls*). The branches are created by a specific enzyme (*gbe*) which transfers a block of 6-7 residues from the end of a linear chain of glucose units to an internal glucose residue by an a-1,6 linkage. De-branching, which is essential for the degradation of glycogen, is catalyzed by a distinct enzyme (*gdb*) which acts on branches containing only four residues, transferring three of them to the end of a linear chain and then hydrolyzing the final residue. The level of glycogen found in a particular situation results from the balance between glycogen synthase and glycogen de-branching activities, resulting in the synthesis and degradation of this compound, respectively. Additionally, both activities are regulated by the action of a glycogen phosphorilase (*gph*). In order to study the role of glycogen metabolism in *Fusarium oxysporum* pathotypic behavior we generated single deletion mutants of the genes D*gnn*, D*gls*, D*gbe*, D*gdb* and D*gph*, and the double mutant D*gph*D*gdb*, by direct targeted replacement. Quantification of glycogen reserves during in vitro growth indicated an increase along the time period (72 h) up 700mg glucose equivalents per mg protein in a pH independent manner. As expected, no detectable glycogen was accumulated in any of three single structural deletion mutants D*gnn5*, D*gls10* or D*gbe17*. By contrast, glycogen levels were 10% higher in the single D*gdb2* and D*gph8*, and the double D*gph8*D*gdb2* mutants in comparison to wild type. Similar hyphal agglutination patterns were observed in the three single mutants D*gnn5*, D*gls10* and D*gbe17* compared to the wild type, whereas those strains affected in glycogen catabolism, D*gdb2*, D*gph8*, and the double mutant D*gph8*D*gdb2*, showed a dramatic reduction in hyphal agglutination. This phenotype did not exactly correlate with conidial anastomosis tube (CAT) formation since all the mutants, except D*gls10*, showed a 40-50% reduction in hyphal fusions. We are currently performing tomato plant infection assays what will help us to gain insight into the role of glycogen metabolism in the virulence of *F. oxysporum*.

554. Identification of chemoattractant compounds from tomato root exudate that trigger chemotropism in *Fusarium oxysporum*. El Ghalid Mennat, David Turra, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain.

Fusarium oxysporum is a soilborne pathogen that causes vascular wilt disease on a wide range of plant species, including tomato (*Solanum lycopersicum*). The host signals that trigger fungal infection are currently unknown. A chemotropic response of *F. oxysporum* towards tomato root exudate was observed using a plate assay that measures directed growth of fungal germ tubes towards chemoattractants. To purifiy the chemoattractant coumpound(s) from tomato root exudate, we applied a series of purification methods including extraction with organic and inorganic solvents, fractionation by size exclusion and ion exchange chromatography. The compound(s) showing chemoattractant activity were found in the hydophilic fraction, had a molecular weight between 30 and 50 kDa and were sensitive to boiling and treatment with proteinase K, suggesting that they correspond to one or several secreted tomato proteins. Polyacrylamide gel electrophoresis of the active fraction revealed multiple protein bands of the expected size, two of which displayed chemoattractant activity when eluted from the gel. Identification of the active protein(s) by LC-ESI-MS is currently ongoing. Identification of the secreted chemoattractant(s) from tomato roots will advance our understanding of the molecular events that trigger fungus-root interactions.

555. TOR-mediated control of virulence functions in the trans-kingdom pathogen *Fusarium oxysporum*. Gesabel Y. Navarro Velasco, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Córdoba, Spain.

Infectious growth of fungal pathogens is controlled by environmental cues, including nutrient status. The soilborne fungus *Fusarium oxysporum* produces vascular wilt disease in more than a hundred different crop species and can cause lethal systemic infections in immunodepressed humans. Previous work showed that the preferred nitrogen source ammonium causes repression of infection-related processes in *F. oxysporum* that could be reversed by rapamycin, a specific inhibitor of the conserved protein kinase TOR. Here we generated mutations in upstream components that should result in constitutive activation of TOR, including null mutants in tuberous sclerosis complex 2 (TSC2), a small GTPase that represses TOR activity, as well as strains expressing a dominant activating allele of the small GTPase Rag (*ragA*^{Q86L}), an activator of TOR. The D*tsc2* mutants and, to a minor extent, the *ragA*^{Q86L} strains showed defects in hyphal growth and colony morphology on several amino acids, as well as decreased efficiency in cellophane penetration and vegetative hyphal fusion. These phenotypes were exacerbated in D*tsc2ragA*^{Q86L} double mutants and could be reversed by rapamycin, suggesting that they are caused by hyperactivation of TOR. The mutants caused significantly lower mortality on tomato plants and on larvae of the animal model host *Galleria mellonella*. These results suggest that TOR functions as a negative regulator of fungal virulence on plant and animal hosts.

556. Components of the urease complex govern virulence of *Fusarium oxysporum* on plant and animal hosts. Katja Schaefer, Elena Pérez-Nadales, Antonio Di Pietro. Departamento de Genética, Universidad de Córdoba, 14071 Cordoba, Spain.

In the soilborne pathogen *Fusarium oxysporum*, a mitogen-activated protein kinase (MAPK) cascade homologous to the yeast filamentous growth pathway controls invasive growth and virulence on tomato plants. Full phosphorylation of Fmk1 requires the transmembrane protein Msb2, a member of the family of signalling mucins that have emerged as novel virulence factors in fungal plant pathogens. A yeast two-hybrid screen for proteins interacting with the Msb2 cytoplasmic tail identified UreG, a component of the urease enzymatic complex. UreG belongs to a set of accessory proteins needed to activate Apo- urease, which converts urea to yield ammonia and carbon dioxide. The *F. oxysporum* genome contains two structural urease genes, *ure1* and *ure2*. Mutants in *ureG* or *ure1* showed reduced growth on urea as the sole carbon and nitrogen source. Lack of urease activity in the mutants resulted in failure to secrete ammonia and to increase the extracellular pH. The D*ureG* mutants caused significantly reduced mortality on tomato plants and on the animal model host *Galleria melonella*, while D*ure1* mutants only showed reduced virulence on tomato plants. Real-time qPCR analysis of key genes involved in nitrogen uptake and assimilation, as well as in the urea cycle, during infectious growth of *F. oxysporum* in *G. melonella* revealed increased transcript levels of arginase, which converts arginine to urea. Our results suggest a role for the urease accessory protein UreG in fungal virulence on plant and animal hosts.

557. Knock-out of the *Fusarium oxysporum* f.sp. *lycopersici* homologs of the DNA-methylation genes *DIM2* and *HP1* does not affect effector gene expression. Charlotte van der Does, Jerom van Gemert, Karlijn Klei, Ido Vlaardingerbroek, Martijn Rep. Molecular plant pathology, SILS, University of Amsterdam, Amsterdam, Netherlands.

In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on a pathogenicity chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is induced upon infection, but the mechanism by which this is regulated is unknown. We noticed that targeted deletion of the effector genes on the pathogenicity chromosome has a particular low rate of succes, when compared to genes on the core genome. Possibly, the pathogenicity chromosome has a more compact (less accessible) chromatin structure. It has been shown that release of chromatin condensation can be a way to regulate gene expression, for example of secondary metabolite gene clusters in *Fusarium* [Reyes-Dominguez et al, FGB 2012]. To test whether DNA methylation in *F. oxysporum* can influence effector expression, knock-outs of *HP1* (heterochromatin protein) and *DIM2* (DNA methylase) were tested for expression of the effector gene *SIX1*. No differences compared to wild-type were observed. Previously it was shown that expression of *SIX1* requires Sge1, a conserved transcription factor encoded in the core genome. Loss of DNA methylation did, however, also not bypass the requirement of Sge1 for *SIX1* expression (in *Dhp1Dsge1* and *Ddim2Dsge1* double mutants). Both *DIM2* and *HP1* are not required for pathogenicity of *F. oxysporum* f.sp. *lycopersici*, and DNA methylation in this strain in general seems to be very low. To obtain more insight in the regulation of effector gene expression we are currently focussing on the potential targets of the transcription factors encoded on the pathogenicity chromosome itself.

558. Mechanistic investigation of *Trichoderma cf. harzianum* SQR-T037 mycoparasitism against *Fusarium oxysporum* f. sp. *cubense* 4, (banana wilt disease). Jian Zhang[1,2], Ruifu Zhang[1,2], Irina S. Druzhinina[3,4], Qirong Shen[1,2]. 1) Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture. 210095, Nanjing, China; 2) Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, 210095, Nanjing, China; 3) Microbiology Group, Institute of Chemical Engineering, Vienna University of Technology Getreidemarkt 9/1665, A-1060 Vienna, Austria; 4) ACIB - Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria.

Besides the effective stimulation of banana growth, the wild strain of *Trichoderma cf. harzianum* SQR-T037 (SQR-T037) is capable to control the soil-born pathogen *Fusarium oxysporum* f. sp. *cubense* 4 (Foc4), the causative agent of banana wild disease . In this work we focused on mechanisms involved in the mycoparasitic attack of SQR-T037 on Foc4. In vitro, in dual confrontation assays, SQR-T037 was able to cover (overgrow) the hyphae of Foc4 what resulted in damage of the cell wall of the pray fungus and its death. At early stages of the interactions, when SQR-T037 hyphae started to combat the colony of Foc4, the droplets of the yellowish exudate putatively secreted by SQR-T037 were observed. The GC-MS analysis identified that the exudate contained almitic and stearic acids, several hydrolytic enzymes (mainly chitinases and proteases) and essential amount of H_2O_2. It allowed us to assume that these compounds play the major role in killing the Foc4. We have detected that hyphae of SQR-T037 indeed accumulated H_2O_2 when it physically made contact with Foc4 and moreover that H_2O_2 suppressed Foc4 growth. The results of transcriptomics analysis of genes putatively involved in the mycoparasitic attack through H_2O_2 or its regulation will be presented.

559. Epichloënin A, a unique siderophore of Epichloae endophytes and its role in restricting fungal growth *in planta*. Natasha Forester[1], Geoffrey A. Lane[1], Iain Lamont[2], Linda J. Johnson[1]. 1) Forage Improvement, AgResearch Limited, Palmerston North, Manawatu, New Zealand; 2) University of Otago, Dunedin, New Zealand.

We have previously shown, through characterisation of a non-ribosomal peptide synthetase gene (NRPS), *sidN*, that the biosynthesis of a novel extracellular ferric iron-chelating siderophore designated as epichloënin A is required for maintaining mutualism of *E. festucae* with its grass host, perennial ryegrass (*Lolium perenne*). We have extended our investigation of the role of fungal siderophores in iron homeostasis through the characterization of other siderophore biosynthetic genes, including *sidA* which encodes a putative L-ornithine N5-oxygenase that catalyses the first enzymatic step in siderophore biosynthesis and *sidC*, encoding a NRPS siderophore synthetase. Using D*sidA*, D*sidC* and D*sidN* mutants we have discovered that *E. festucae* synthesises two siderophores, epichloënin A that requires SidN and an intracellular siderophore, ferricrocin (FC) that requires SidC; production of both siderophores is totally abolished in D*sidA* mutants. In contrast to D*sidN* mutants, D*sidC*-infected plants showed very little phenotypic consequences due to loss of FC, and this is likely to be due to functional redundancy of epichloënin A. The levels of iron-bound epichloënin A inside the cell are significantly higher than FC, suggesting that epichloënin A acts both as an extracellular and intracellular siderophore. While investigating the influence of iron on the siderophore mutants *in planta* with hydroponically supplied nutrients, we unexpectedly demonstrated that iron exacerbates rather than complements the D*sidN* mutant phenotype. We observed increases in fungal proliferation, production of dense mycelial mats, and increased plant stunting. In infected plants, compared to wild-type (WT), there was also an increased uptake of iron in D*sidN* cells by reductive iron assimilation. Analyses in culture indicated that in the D*sidN* mutant more iron was stored (in FC and in vacuoles) than in WT fungi, suggesting that D*sidN* has increased access to iron relative to WT. However, ultra-structural studies of D*sidN* hyphae in stunted plants (that were not associated with the host vasculature) suggested that D*sidN* hyphae were often non-viable, implicating resource exhaustion from inappropriate growth and misuse of available iron. We therefore propose that epichloënin A is a multitasking siderophore specialising in iron sequestration to moderate cellular iron supply.

560. Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots. Nils OS Högberg[1], Anna Rosling[1], Annegret Kohler[2], Martin Francis[2], Stenlid Jan[1]. 1) Department of Forest Mycology, BioCenter, SLU, Uppsala, Sweden; 2) INRA, Nancy, France.

Recently it has been shown, with a Comparative genomic perspective, that brown rot and mycorrhiza fungi have evolved from white rot ancestors. Wood is a composite material composed of lignin, cellulose and hemicellulose. White rot fungi are able to degrade all of these components with a combination of carbohydrate active and oxidative enzymes. During the course of evolution brown rot and mycorrhiza have lost most of the genes in these gene families. Nevertheless, brown rot fungi are efficient wood decomposers that degrade cellulose and hemicellulose by means of hydroxyl radical production and remaining carbohydrate active enzymes. The family Boletales includes both brown rot fungi and mycorrhiza and it is tentative to speculate that there has been a parallel evolution of these ecological strategies. Here we test the effect of infecting pine roots with the brown rot fungus Serpula lacrymans. The interaction was neutral since plant growth was not stimulated but not reduced either. The fungus formed a mantle around the pine roots but not the Hartig net that is typical for ectomycorrhiza. Fungal gene expression was compared with the wood decay transcriptome. 1250 genes were more than two-fold upregulated compared to a glucose medium control. A large proportion of the upregulated genes (62 %) are unknown. Carbohydrate active genes represent only 3% of this gene set and genes with oxidoreductase activity, including monoxygenases represent 4% of the upregulated genes. This is considerably lower compared to saprotrophic growth on wood where carbohydrate active enzymes accounted for 26% and oxidative enzymes for 19% which dominated the gene expression on wood. Gene expression for genes involved in transportation was about the same, around 10% in this experiment and under wood decomposition. Several genes that indicate an interaction with a host were also upregulated. In conclusion, gene expression was markedly different between a glucose medium, wood decomposition and growth on pine roots. This may be a signal of symbiosis, the effect on pine seedling growth was neutral. Thus we cannot conclude if the interaction is beneficial or negative to the host.

561. You turn me on: *Pyrenophora tritici-repentis* genes differentially regulated early during infection of wheat. V. A. Manning[1], I. Pandelova[1], L. M.

Ciuffetti[1,2]. 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; 2) Center of Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Pyrenophora tritici-repentis (*Ptr*) is a necrotrophic fungal pathogen of wheat causal to the disease tan spot and host-selective toxins (HSTs) produced by *Ptr* are the primary factors that contribute to virulence. One of these HSTs, Ptr ToxA, is a necrosis-inducing, proteinaceous HST that is also present in the wheat pathogen *Stagonospora nodorum*. Ptr ToxB is a chlorosis-inducing, proteinaceous HST produced by *Ptr* with active orthologues expressed in the brome grass pathogen, *Pyrenophora bromi.* Despite the presence of active, orthologous HSTs produced by other fungi, *Ptr* appears to have an advantage in some wheat growing regions in the world, surpassing in disease relevance the other necrotrophic pathogens that contain orthologous HSTs. To begin to understand the molecular mechanisms that underlie this advantage, we used RNA-seq-based transcriptome analysis to identify *Ptr* genes that are differentially regulated *in planta* early in the disease cycle (at thirty hours post-infection) as compared with those genes that are expressed in culture. Functional annotations of the differentially expressed-*in planta* transcripts reflect the requirements of the pathogen for host penetration, cell wall degradation and the need to counteract the host response to infection; these include CAZymes, peptidases, transporters, and loci with predicted oxido-reductase activities including peroxidases. In addition, putative secondary metabolite clusters and *Ptr*-specific proteins are also differentially regulated. These findings provide the basis for understanding the roles of these proteins in virulence and the possibility of revealing common transcriptional regulatory elements activated by interactions with the host.

562. Characterisation of genes in Quantitative Trait Loci affecting virulence in the basidiomycete *Heterobasidion annosum* s.l. Ake Olson, Yang Hu, Inga Bödeker, Malin Elfstrand, Mårten Lind, Jan Stenlid. Dept Forest Mycology/Pathology, SW Univ Agricultural Sci, Uppsala, Uppsala, Sweden.

Heterobasidion annosum sensu lato (s.l.), is a devastating root rot pathogen on conifers present all over the northern hemisphere that causes losses of 500 of million Euro per year for forest owners. The *H. annosum* s.l. consists of five phylogenetic distinct species with different but overlapping host preferences. The genome sequence of one isolates from *H. irregulare* and *H. occidentale* preferentially infecting pine and spruce species, respectively have been obtained. Analysis of the progeny of a genetic cross of the sequenced isolates resulted in a genetic linkage map of 15 groups representing almost the complete chromosome set-up. These groups have been aligned and anchored to the physical map of *H. irregulare*. Quantitative trait loci (QTL) for virulence on one-year-old *Pinus sylvestris* and two-year-old *Picea abies* seedlings were identified and positioned on the map allowing a straight forward identification of virulence candidate genes. Gene content and sequence divergence of the QTL regions will be presented. Detailed expression analysis of virulence candidate genes with Q-PCR and protein localisation with immunohistochemistry will deduce their role during infection.

563. Elevation of FPP synthase activity in *Trichoderma atroviride* results in higher biocontrol abilities. Sebastian Graczyk, Urszula Perlinska-Lenart, Wioletta Gorka-Niec, Patrycja Zembek, Sebastian Pilsyk, Grazyna Palamarczyk, Joanna S. Kruszewska. Laboratory of Fungal Glycobiology, Institute of Biochemistry and Biophysics, Warsaw, Poland.

In this study we present a new method to obtain the *Trichoderma* strains with enhanced antifungal and biocontrol activities. The method is based on the increase synthesis of the mevalonate pathway products. In this pathway some metabolites are produced such as terpenoids, trichodermin, harzianum A, mycotoxin T2, lignoren, ergokonin A and B and viridin which are known from their antifungal and antibacterial activities. All these compounds are synthesized from farnesyl pyrophosphate (FPP) which is itself synthesized by farnesyl pyrophosphate synthase encoded by *ERG20* gene. FPP is also a substrate for dolichol and ergosterol production which are indirectly engaged in antimicrobial action. In this study we increased production of FPP in *T. atroviride* by overexpression of the yeast *ERG20* gene. We based on the assumption that the increased activity of FPP synthase would stimulate production of all products of the mevalonate pathway. Five transformants showed higher activity of FFP synthase. Simple screening of the hydrolytic properties of the *ERG20* transformants revealed that they grew faster on plates with polycarbohydrates as carbon sources. Detailed studies showed higher cellulotytic and chitinolytic activity of enzymes secreted to the cultivation medium by the transformed strains. Antifungal activity was examined by cultivation of plant pathogen *Rhizoctonia solani* in the atmosphere of volatiles liberated by the transformants and the control. We also analyzed growth of *Pythium ultimum* on plates which were previously overgrown by *Trichoderma* strains and were filled with their metabolites. Both experiment showed significantly stronger inhibition of growth of the pathogens by the transformed strains compared to the control. Since the above experiments revealed enhanced antifungal properties of the transformed strains we performed plant tests using the bean *Phaseolus vulgaris L.*. Transformed strains increased both, the germination rate and the size of plants growing in soil infected by *Pythium ultimum* compared to the control strain. To conclude, an increased activity of the mevalonate pathway caused higher activity of hydrolytic enzymes and increased production of volatiles and secondary metabolites and that way boosted antifungal and biocontrol activities of the *Trichoderma ERG20* transformants.

564. The life history of Ramularia collo-cygni. Maciej Kaczmarek[1,2], James Fountaine[1], Adrian Newton[3], Nick Read[2], Neil Havis[1]. 1) Crop and Soil Research, Scotland's Rural College, Edinburgh, United Kingdom; 2) Institute of Cell Biology, University of Edinburgh, Edinburgh, United Kingdom; 3) Cell and Molecular Sciences, The James Hutton Institute, Dundee, United Kingdom.

The filamentous fungus Ramularia collo-cygni causes the late season disease of spring and winter barley called Ramularia Leaf Spot (RLS). It has become an increasingly important problem for European farmers in the past decade and has recently been reclassified as a major disease of barley in the UK. The lack of apparent varietal resistance to the disease has led to significant amounts of fungicide being applied to crops in north western and central Europe in order to maintain green leaf area and prevent significant yield loss. These factors have contributed to an increasing focus on achieving a better understanding of the fundamental biology of this elusive pathogen in order to develop more successful strategies of RLS management. Therefore, disease development throughout the life cycle of the host barley plant has been analysed by the employment of transgenic R. collo-cygni isolate, expressing the GFP reporter molecule, and confocal microscopy. We have been able to examine the previously uncharacterised seed-borne stage and illustrate the mode of fungal transmission into barley seedlings. We have also analysed the potential sexual reproduction in the fungus by utilising a range of correlative techniques, such as cryo-scanning electron microscopy, confocal microscopy and light microscopy. Here we describe for the first time the nature of speculated spermogonial stage called Asteromella and in addition, present preliminary evidence suggesting the existence of a perfect stage that, if functional, could resemble closely related Mycosphaerella species.

565. Mechanical stress sensing in *Epichloë* fungal symbionts during colonization of grasses. Kahandawa G.S.U Ariyawansa[1], Rosie E. Bradshaw[2], Neil A.R. Gow[3], Nick D. Read[4], Richard D. Johnson[1], Duane P. Harland[5], Christine R. Voisey[1]. 1) AgResearch, Grasslands Research Centre, Palmerston North, New Zealand; 2) BioProtection Centre, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 3) School of Medical Sciences, University of Aberdeen, United Kingdom; 4) Institute of Cell Biology, University of Edinburgh, United Kingdom; 5) AgResearch, Lincoln Research Centre, Christchurch, New Zealand.

Epichloë festucae is an agronomically-important endophytic fungus that grows symbiotically within the intercellular spaces of temperate grass species

such as *Lolium perenne* and *L. arundinaceum*. Colonization of host seedlings by *E. festucae* occurs when hyphae in the shoot apex invade developing host leaves and extend via intercalary hyphal growth, a highly unusual mechanism of division and extension in non-apical compartments. We hypothesise that intercalary hyphal growth is stimulated by mechanical stretch imposed by attachment of hyphae to elongating host cells, and that this stress is sensed by mechano-sensors located on the hyphal membranes. Genome analysis revealed that homologues of known mechano-sensors in *Saccharomyces cerevisiae* such as Mid1 (a stretch activated calcium ion channel), Wsc1 and Mid2 (cell wall integrity sensors) are present in the *E. festucae* genome. Gene replacement studies of *mid1* and *wsc1* in *E. festucae* reduced radial growth rate in axenic culture confirming the role of both genes in hyphal growth. In axenic culture both *Dwsc1* and *Dmid1* mutants were sensitive to fungal cell wall modifiers such as Calcofluor White, supporting their role in cell wall integrity. Preliminary plant infection studies with *Dwsc1* and *Dmid1* mutants revealed a hyper-branched unsynchronized growth pattern within the host (*Lolium perenne*), and *Dwsc1* also caused severe stunting in most plants suggesting a disruption in the symbiosis. A technique to stimulate intercalary growth under *in-vitro* conditions through mechanical stretch is being optimised to test the ability of Mid1, Wsc1 and Mid2 to sense mechanical stress and initiate intercalary growth.

566. *Aspergillus flavus* hypertrophy and hyphal entry by *Ralstonia solanacearum* is mediated by bacterial type three secretion system function. Joe E Spraker[1], Nancy P Keller[2]. 1) Plant Pathology, University of Wisconsin Madison, Madison, WI; 2) Bacteriology, University of Wisconsin Madison, Madison, WI.

Fungi and bacteria are two of the primary pathogens of plants, often infecting the same crops, however shockingly little is known of how these organisms interact independently of plant hosts. In examining the interaction between two economically important pathogens of peanut, Aspergillus flavus and Ralstonia solanacearm, a fungus and bacterium, respectively, we've shown that fungal hypertrophy is induced and that the bacterium is capable of entering these cells. The hypertrophic cells were imaged using calcofluor staining to show chitin cell wall structure. Bacterial invasion of these structures was demonstrated using confocal microscopy of GFP labeled bacteria. Further, we demonstrate that bacterial mutants deficient in type three secretion systems are incapable of eliciting the fungal hypertrophic response by culturing virtually isogenic bacterial type three secretion mutants. This is the first report of a well-known plant pathogenic bacterium eliciting fungal hypertrophy and invading hyphal cells. Current research is aimed at finding bacterial effectors that may be facilitating this interaction and elucidating their mode of action.

567. Vegetative hyphal fusion in epichloae endophytes. Jun-ya Shoji, Nikki D. Charlton, Sita R. Ghimire, Jin Nakashima, Kelly D. Craven. Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

Vegetative hyphal fusion establishes the interconnection of individual hyphal strands into an integrated network of a fungal mycelium. It is suspected that vegetative hyphal fusion plays many important roles such as in nutrient translocation, intramycelial signaling, and emergence of genetic diversity via horizontal gene / chromosome transfer or interspecific hybridization. However, experimental support for these suspected roles is still largely lacking. To investigate the role of hyphal fusion in fungal endophytes of epichloae, which form mutualistic symbiosis with grass hosts, we generated mutant strains lacking *sftA*, an ortholog of the hyphal fusion gene *so* in *Epichloë festucae*. The *E. festucae DsftA* mutant strains grew like the wild-type strain in culture but with reduced aerial hyphae, and completely lacked hyphal fusion. The most striking phenotype of the *E. festucae DsftA* strain was that it failed to establish a mutualistic symbiosis with the tall fescue plant host (*Lolium arundinaceum*), and instead, killed the host plant within two months after initial infection. This suggests that hyphal fusion may have an important role in the establishment / maintenance of fungal endophyte-host plant mutualistic symbiosis. To further investigate the importance of hyphal fusion in epichloae, frequency of hyphal fusion was quantified in different epichloae endophytes including sexual isolates, asexual interspecific hybrids and asexual non-hybrids. A majority of sexual epichloae underwent frequent hyphal fusion, whereas hyphal fusion was less frequently found in asexual epichloae. Moreover, hyphal fusion was less common in asexual non-hybrid epichloae compared to asexual hybrids. Thus, it appears that the ability to undergo hyphal fusion correlates with the presence of the sexual cycle, and the hybrid status of epichloae endophytes. Overall, our data provide evidence for the importance of hyphal fusion in establishment / maintenance of mutualistic symbiosis, and evolution of epichloae endophytes.

568. Oxygen and the stomatal cue: Dissecting stomatal tropism in *Cercospora zeae-maydis*. R. Hirsch, B. Bluhm. Department of Plant Pathology, University of Arkansas Division of Agriculture, Fayetteville, AR.

Cercospora zeae-maydis causes grey leaf spot of maize, one of the most widespread and destructive foliar diseases of maize in the world. Stomatal infection is a critical, yet poorly defined, component of pathogenesis in *C. zeae-maydis*. At the onset of infection, the fungus senses and grows towards maize stomata, and then breaches the leaf surface by producing appressoria over stomatal pores. Directed growth toward distant stomata during infection led us to hypothesize that *C. zeae-maydis* responded to an unknown chemical cue emanating from stomata. To elucidate mechanisms underlying infectious development in *C. zeae-maydis*, particularly stomatal tropism, a series of histological experiments were performed with epi-florescent and confocal microscopy. Upon sensing maize stomata, *C. zeae-maydis* either reoriented hyphal tip growth towards stomata, or initiated new hyphae originating from right-angle branches in close proximity to stomata. Hyphae exhibiting stomatal tropism were linear and lacked branches. On topographically accurate acrylic leaf replicas, *C. zeae-maydis* did not display stomatal tropism and failed to form appressoria upon encountering artificial stomata, which indicated that thigmotropic cues were not sufficient to elicit pre-penetration infectious development. However, in non-host interactions, *C. zeae-maydis* exhibited stomatal tropism and retained the ability to form appressoria over stomata, which suggested that a chemical cue emanating from stomata elicited a chemotropic response in the fungus. Stomatal tropism and appressoria formation in *C. zeae-maydis* were impaired when atmospheric oxygen levels were disturbed, implicating the role of oxygen sensing in pathogenicity. This study characterized stomatal tropism during infection of maize by *C. zeae-maydis*, directly implicated oxygen sensing as a component of pathogenicity, and provides a quantitative framework through which to study foliar pathogenesis and host/pathogen interactions in related systems.

569. Host colonisation processes by symbiotic epichloid fungi are regulated through cAMP. Christine R. Voisey[1], Damien J. Fleetwood[2], Linda J. Johnson[1], Gregory T. Bryan[1], Wayne R. Simpson[1], Michael J. Christensen[1], Suzanne J.H. Kuijt[1], Kelly Dunstan[1], Richard J. Johnson[1]. 1) Forage Biotechnology, AgResearch, Palmerston N, New Zealand; 2) School of Biological Sciences, The University of Auckland, Auckland 1142, New Zealand.

The fungal symbiont, *Epichloë festucae*, colonises leaves of host grasses by ramifying through the shoot apical meristem (SAM) of the seedling, and then infecting the leaf primordia. Hyphal infection of the SAM is dependent on apical growth, however after primordia have formed, leaf tissues undergo a phase of intercalary expansion, which the fungus, attached to host cells, must recapitulate to remain intact. *E. festucae* hyphae entering the leaf expansion zone switch from apical to intercalary growth, and extend in synchrony with the host until the leaf tissues mature. How colonising symbiotic fungi accommodate the complexities of the plant developmental programme is currently unclear. Since cAMP signalling is often required for host colonisation by fungal pathogens, we disrupted the cAMP cascade by insertional mutagenesis of the *E. festucae* adenylate cyclase gene (*acyA*). Consistent with reports

on other fungi, disruption mutants had a slow radial growth rate in culture, and colonies were highly compact relative to controls. Furthermore, the hyphae were convoluted and hyper-branched suggesting that apical dominance had been disrupted. Nitro blue tetrazolium straining of hyphae showed that cAMP disruption mutants were impaired in their ability to synthesise superoxide indicating that cAMP signalling is important for the production of ROS in culture in this species. This defect was reversed by re-insertion of a functional wild type *acyA* gene into mutant strains. Despite significant defects in hyphal growth and ROS production in culture, *E. festucae* D*acyA* mutants were infectious and capable of forming symbiotic associations with grasses, albeit at a lesser infection frequency than wild type. Plants infected with *E. festucae* D*acyA* mutants were indistinguishable from controls. However, as in culture, microscopic evidence showed that the mutant strains within the host were hyper-branched, and host tissues heavily colonised, indicating that the tight regulation over hyphal growth normally observed in developing and mature host tissues requires a functional cAMP signalling cascade. Further research is currently underway to understand how cAMP affects the hyphal growth transitions undertaken during host colonisation, particularly at the level of the cell cytoskeleton and hyphal cell wall synthesis.

570. Role of VCP1 and SCP1 proteases in the mutitrophic behaviour of the nematophagous fungus *Pochonia chlamydosporia*. Nuria Escudero[1], Christopher R. Thornton[2], Luis Vicente Lopez-Llorca[1]. 1) Laboratory of Plant Pathology, Multidisciplinary Institute for Environment Studies (MIES) Ramón Margalef. University of Alicante, Alicante, SPAIN; 2) Food Security and Sustainable Agriculture, Biosciences, College of Life & Environmental Sciences, University of Exeter, Exeter. UK.

Pochonia chlamydosporia (Goddard) Zare and Gams is a fungal parasite of female nematodes and eggs, which has been widely studied as a biological control agent of cyst and root-knot nematode egg-shells. The nematode egg-shell is formed by several layers, including a chitinous layer composed of a protein matrix embedding chitin microfibrils. Extracellular enzymes, such as serine porteases (e.g. VCP1), secreted by egg-parasitic nematophagous fungi are known to play an important role in egg infection. SCP1, a recently reported serine carboxypeptidase from *P. chlamydosporia* was found during plant root endophytic colonisation by the fungus, its role in eggs parasitisim is unknown. We have investigated the role of VCP1 and SCP1 proteases in the mutitrophic behaviour of the nematophagous fungus *Pochonia chlamydosporia* using immunological approaches using antiVCP1 and SCP1 polyclonal antibodies, these were raised against synthetic peptides of both proteases. ELISA and immunofluorescence have confirmed the production of both proteases when *Meloidogyne javanica* eggs were used as inducer. *P. chlamydosporia* under starvation condition (water) also expressed both proteases. It seems that the signal of SCP1 was more intense than of VCP1 under most conditions tested (eggs, protein substrate and starvation). Using proteomic, chitosan was previously found in our lab to induce VCP1 in *P. chlamydosporia* liquid cultures. Consequently, we have also evaluated the amount of VCP1 and SCP1 in media with chitosan, to quantify the production of these proteases under multitrophic conditions. This study is casting light into the molecular aspects of the multitrophic behaviour of *P. chlamydosporia*. This will help to understand the biocontrol potential of the fungus and open new biotechnological applications.

571. Cellular development integrating primary and induced secondary metabolism in the filamentous fungus *Fusarium graminearum*. Jon Menke[1], Jakob Weber[2], Karen Broz[3], H. Corby Kistler[1,3*]. 1) Department of Plant Pathology, University of Minnesota, St. Paul, USA; 2) Molekulare Phytopathologie, Universität Hamburg, Germany; 3) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

Several species of the filamentous fungus *Fusarium* colonize plants and produce toxic small molecules that contaminate agricultural products, rendering them unsuitable for consumption. Among the most destructive of these species is *F. graminearum*, which causes disease in wheat and barley and often contaminates the grain with harmful trichothecene mycotoxins. Induction of these secondary metabolites occurs during plant infection or in culture in response to chemical signals. Here we report that trichothecene biosynthesis involves a complex developmental process that includes dynamic changes in cell morphology and the biogenesis of novel subcellular structures. Two cytochrome P-450 oxygenases (Tri4p and Tri1p) involved in early and late steps in trichothecene biosynthesis were tagged with fluorescent proteins and shown to co-localize to vesicles we call “toxisomes.” Toxisomes, the inferred site of trichothecene biosynthesis, dynamically interact with motile vesicles containing a predicted major facilitator superfamily protein (Tri12p) previously implicated in trichothecene export and tolerance. The immediate isoprenoid precursor of trichothecenes is the primary metabolite farnesyl pyrophosphate. When cultures are shifted from non-inducing to trichothecene inducing conditions, changes occur in the localization of the isoprenoid biosynthetic enzyme HMG CoA reductase. Initially localized in the cellular endomembrane system, HMG CoA reductase increasingly is targeted to toxisomes. Metabolic pathways of primary and secondary metabolism thus may be coordinated and co-localized under conditions when trichothecene synthesis occurs.

572. DNA double-strand breaks generated by yeast endonuclease *I-Sce* I induce ectopic homologous recombination and targeted gene replacement in *Magnaporthe oryzae*. T. Arazoe[1], T. Younomaru[1], S. Ohsato[1], T. Arie[2], S. Kuwata[1]. 1) Meiji University, Kanagawa, Japan; 2) Tokyo University of Agriculture and Technology, Tokyo, Japan.

The filamentous fungus *Magnaporthe oryzae* causes the rice blast disease that is one of the most destructive fungal diseases of cultivated rice plants. To control this fungal disease, many resistant genes have been introduced into cultivated rice germplasm, however, breakdowns of the resistance often occur within several years by rapid evolution of the fungus. Therefore, studies on the evolutionary mechanisms of the fungus are important for elucidation of the rapid evolution. We set out a novel detection/selection system of DNA double-strand breaks (DSBs)-mediated ectopic homologous recombination (HR) that is one of the evolutionary mechanisms. The system consists of two nonfunctional *yellow fluorescent protein* (*YFP*)/*blasticidin S deaminase* (*BSD*) fusion genes as a donor and a recipient, and a yeast endonuclease *I-Sce* I gene as a DSB-inducer. In this system, ectopic HR can be detected and selected by restorations of YFP fluorescence and blasticidin S (BS)-resistance at a single cell level. These donor and recipient genes were simultaneously integrated into the *M. oryzae* genome and transformed lines were isolated. In the absence of the DSB-inducer, transformed lines showed relatively low frequencies of HR events (>2.1%). On the other hand, by integration of the DSB-inducer gene into transformed lines, we could observe the frequencies of DSB-mediated HR raising up to ~40%. This result clearly showed that DSB into a certain gene induce ectopic HR events between the gene and its homologs. Accordingly, we further applied *I-Sce* I mediated DSB for TGR in *M. oryzae*. To detect TGR, we constructed simple system using donor and recipient genes. The recipient gene was integrated into the *M. oryzae* genome and transformed lines were isolated. To recipient gene integrated lines, the donor gene was introduced and restorations of YFP fluorescence and BS-resistance were evaluated. As we expected, the TGR frequencies were increased at least 37-folds by *I-Sce* I co-transformation as compared with those obtained without *I-Sce* I. This result provides a new method using DSB for improving the TGR frequency in *M. oryzae*. Taken together, it is strongly suggested that DSBs can drive genomic rearrangement and accelerate pathogenic variability in *M. oryzae* through the ectopic HR between homologous sequences such as transposable elements and avirulence genes.

573. Investigation of the *Magnaporthe oryzae* proteome and phosphoproteome during appressorium formation. William L. Franck[1], Emine Gokce[2], Yeonyee Oh[1], David C. Muddiman[2], Ralph A. Dean[1]. 1) Plant pathology, NC State University, Raleigh, NC; 2) W.M. Keck FT-ICR Mass Spectrometry

Laboratory, NC State University, Raleigh, NC.

Magnaporthe oryzae, the causative agent of rice blast disease, infects plant leaves via formation of an appressorium which facilitates penetration of the leaf surface. In an effort to better understand the physiological changes accompanying the earliest stages of infection-related development, a nano-LC MS/MS-based global proteomics examination of conidial germination and cAMP-induced appressoria formation was undertaken at four distinct developmental time points resulting in the identification of 3200 proteins. Label free quantification by spectral counting identified 591 proteins whose relative abundance changed during germination. Furthermore, treatment of germinating conidia with cAMP to induce appressorium formation resulted in the identification of 493 proteins whose relative abundance changed compared to untreated samples. In developing appressoria, changes in cell wall modifying, transport, extracellular and plasma membrane localized proteins were observed. Proteomic analysis of a *M. oryzae* cAMP-dependent protein kinase A (cpkA) mutant defective in appressorium formation following treatment with cAMP identified a subset of proteins whose regulation is dependent on cAMP signaling. A comparison of proteome and transcriptome data revealed little correlation between protein and transcript regulation. Finally, to better define the role of protein phosphorylation and the CPKA protein kinase during the development of appressoria, the phosphoproteome of *M. oryzae* is being investigated. To date, a total of 980 phosphoproteins have been identified from conidia and experiments designed to identify changes in protein phosphorylation during appressorium formation are in progress.

574. Characterization of the binding site and downstream targets of the *MST12* transcription factor in *Magnaporthe oryzae*. Guotian Li, Guanghui Wang, Jin-Rong Xu. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases on rice. In *M. oryzae*, appressorium formation is regulated by *PMK1* mitogen-activated protein kinase (MAPK) pathway. Its orthologs are conserved a wide array of plant pathogenic fungi for regulating different plant infection processes. In *M. oryzae*, one of the transcription factors functioning downstream from Pmk1 is Mst12 that is essential for appressorium penetration and invasive growth. The *MST12-GFP* transformant showed strongest GFP signals in appressoria and invasive hyphae. Pmk1 weakly interacted with Mst12 in yeast two-hybrid assays. Overexpression of *MST12* failed to complement the defects of the *pmk1* mutant, suggesting that the activation of Mst12 by Pmk1 was required for its function. Site-directly mutagenesis analysis indicated that the MAPK-docking region and phosphorylation site were essential for the function of *MST12*. However, mutant alleles of *MST12* with point mutations in any one of the two tandem zinc finger domains were still partially functional, indicating their overlapping or redundant functions. Expression of *MST12* functionally rescued the invasive growth defects of the yeast *ste12* mutant and EMSA analyses suggested that Mst12 had a binding site similar to the PRE sequence recognized by Ste12. During appressorium formation, transcription levels of 222 genes were found to be significantly altered in the *mst12* mutant ($P<0.005$). Ten of these downstream targets of *MST12* with putative PREs in their promoters were selected for functional characterization. Data on phenotype characterization of the resulting mutants and promoter element analysis will be presented.

575. CorA Magnesium transporters are key regulators of growth and pathogenicity in the rice blast fungus M. oryzae. Md Hashim Reza, J. Manjrekar, Bharat B. Chattoo. Microbiology and Biotechnology Centre, Genome Research Centre,MS University, Baroda, Vadodara, India.

Magnaporthe oryzae, the causative organism of rice blast disease, infects cereal crops and grasses at various stages of plant development. Thus, a comprehensive understanding of the fungal metabolism and its implications on pathogenesis is a must to countering this devastating crop disease. As a co-factor for functionally diverse enzymes, Mg2+ regulates a variety of biological processes. Magnesium transporters have been shown to be essential for viability of S. cerevisiae and some bacterial pathogens. We present the molecular identity, function and regulation of magnesium (CorA) transporters and their role in growth and pathogenicity of the filamentous fungal pathogen, M. oryzae. Complementation analysis in S. cerevisiae showed that magnesium transporters, MoALR2 and MoMNR2, have overlapping functions. Further, we have shown that reduction of Mg2+ transporters using a knock-down approach leads to decrease in intracellular magnesium levels, indicating that these transporters play a crucial role in Mg2+ homeostasis of M. oryzae. Functionally, the mutants display defects in surface hydrophobicity, cell wall stress tolerance, sporulation, appressorium (infective structure) formation and infection, making MoALR2 and MoMNR2 indispensible for growth and pathogenesis of the rice blast fungus M. oryzae. MoMPS1 (MAPK) and MoMPK1 (MAPK) driven signaling pathways have been shown to be essential for cell wall integrity, conidiogenesis, appressorium formation and invasive growth. Expression analysis by qPCR of MoPMK1 (MAPK) and MoMPS1 (MAPK) signalling pathways, core to the above mentioned phenotypes, showed deviation from the wild type. We also detected corresponding differences in downstream MoMPS1 driven Cell Wall Integrity (CWI) signalling and the MoPMK1-regulated hydrophobin, MoMPG1, expression.

576. *Magnaporthe oryzae* AVR-Pia protein: induction of resistance reaction in *Pia* rice by the recombinant AVR-Pia and preparation of anti-AVR-Pia antibody. Y. Satoh[1], T. Ose[2], R. Terauchi[3], T. Sone[1]. 1) Graduate school of Agriculture, Hokkaido Univ., Sapporo, Hokkaido, Japan; 2) Research faculty of Pharmacology, Hokkaido Univ., Sapporo, Hokkaido, Japan; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, Japan.

The avirulence gene *AVR-Pia*, which induces hypersensitive reaction (HR) of rice cultivars with the resistance gene *Pia* was isolated from *Magnaporthe oryzae* strain Ina168 (Miki *et al*., 2009). The qRT-PCR analysis revealed that *AVR-Pia* expression was started from 24 hours post inoculation on the rice leaf. Furthermore, AVR-Pia protein localization to biotrophic interfacial complex (BIC: Khang *et al*., 2010) was observed using AVR-Pia::eGFP fusion protein in compatible rice leaf sheath cells. On the other hand, the functional detail of AVR-Pia protein during infection was not understood. In order to analyze the function of AVR-Pia protein, the recombinant AVR-Pia protein was purified from *E. coli*. Recombinant AVR-Pia was revealed to induce HR-like browning spots when it was infiltrated into *Pia* rice leaf, suggesting the activity to trigger the host's resistance reaction. An anti-AVR-Pia antibody was prepared with the recombinant protein, and its validity was investigated by Western blotting. Native AVR-Pia was detected from total soluble protein extracted from inoculated pia rice leaf sheath, and the MW of AVR-Pia was estimated as 7.4 kDa, corresponding to AVR-Pia w/o signal peptide. This result suggested that recombinant AVR-Pia has similar structure to the native AVR-Pia, and confirmed the affinity of antibody to native AVR-Pia. Quantification of secreted AVR-Pia by *M. oryzae* during infection was performed with anti-AVR-Pia. It was revealed that approx. 1.5 ppm (relative to total soluble protein of Ina168-inoculated rice sheath) of AVR-Pia was secreted during infection. These facts suggest that the *Pia* rice can recognize AVR-Pia at lower concentration than 1.5 ppm and trigger the immune response at the early stage of blast infection.

577. Homologous recombination causes the spontaneous deletion of *AVR-Pia* in *Magnaporthe oryzae*. T. Sone, S. Takeuchi, S. Miki, Y. Sato, K. Ohtsuka, A. Abe, K. Asano. Res Faculty Agriculture, Hokkaido Univ, Sapporo, Japan.

AVR-Pia, an avirulence gene in the genome of the rice blast fungus *Magnaporthe oryzae*, triggers a hypersensitive reaction in rice cultivars harboring the resistance gene *Pia*. The copy number of *AVR-Pia* was revealed to vary from one to three among *M. oryzae* isolates avirulent to *Pia* rice, and three copies of the gene were located on a single chromosome in strain Ina168, from which the gene was originally cloned. The spontaneous *AVR-Pia* mutant originated from Ina168m95-1, which lacks the *AVR-Pia* gene, and was therefore used to elucidate the molecular mechanism of the deletion of all three

copies of *AVR-Pia*. Screening and analysis of cosmid clones indicated that two copies of the DNA-type transposon *Occan* (*Occan*^{9E12} and *Occan*^{3A3}) were located on the same chromosome and three copies of *AVR-Pia* were located in between the two *Occan* elements. Ina168m95-1 contains a conserved *Occan* element, named *Occan*$^{m95\text{-}1}$, between sequences homologous to the 5'-flanking region of *Occan*^{3A3} and the 3'-flanking region of *Occan*^{9E12}. In addition, sequence polymorphisms indicated a homologous recombination between *Occan*^{3A3} and *Occan*^{9E12}, which resulted in *Occan*$^{m95\text{-}1}$. Based on these observations, we propose the hypothesis that homologous recombination in the two *Occan* elements leads to the deletion of *AVR-Pia* in Ina168m95-1.

578. The interactome of pathogenicity factors in the rice blast fungus *Magnaporthe oryzae*. Xiaoying Zhou[1], Yang Li[1], Keerthi Jayasundera[2], Anton Iliuk[2], Andy Tao[2], Jinrong Xu[1]. 1) Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN; 2) Dept. of Biochemistry , Purdue University, West Lafayette, IN.

Rice blast is a disease of significant economic impact worldwide and a model system for studying fungal-plant interactions. To date, over 100 pathogenicity factors have been identified in Magnaporthe oryzae. However, there is only limited knowledge about their relationships. To better understand molecular mechanisms regulating plant infection processes, it is critical to identify protein-protein interaction networks important for pathogenesis. In this study, we characterized the interactome of selected pathogenicity-related proteins. The affinity purification and proteomics approaches were used to identify proteins that interact with over 60 known pathogenicity factors, including components of important signaling pathways. Protein-protein interaction maps were established for these pathogenicity factors based on affinity purification data and information about their orthologs in yeast. Co-immunoprecipitation, BiFC, or yeast two hybridization assays were used to verify the interactions of selected genes. For a number of pathogenicity factor-interacting genes, gene knock-out mutants were generated to determine their functions in pathogenesis. To our knowledge, results from this study represent the first study of protein-protein interaction networks of pathogenicity factors in plant pathogenic fungi in *M. oryzae*.

579. Interaction between phenolic and oxidant signaling in *Cochliobolus heterostrophus*. Benjamin A Horwitz[1], Samer Shalaby[1], Olga Larkov[1], Mordechai Ronen[2], Sophie Lev[3]. 1) Department of Biology, Technion - IIT, Haifa, Israel; 2) Department of Plant Science, Tel Aviv University, Ramat Aviv, Israel; 3) Centre for Infectious Diseases and Microbiology, University of Sydney at Westmead Hospital, Westmead, NSW 2145, Australia.

The transcription factor ChAP1 is an ortholog of yeast YAP1 in the maize pathogen *Cochliobolus heterostrophus*. ChAP1 migrates to the nucleus upon exposure to oxidative stress, inducing antioxidant genes such as thioredoxin and glutathione reductase [1]. ChAP1 also localizes to nuclei on contact with the leaf and during invasive growth. Though reactive oxygen species are encountered on the host, ChAP1 nuclear retention can occur without oxidative stress. One of the signals responsible is provided by phenolic compounds [1-3]. Using a genetically-encoded ratiometric reporter of the redox state, we showed that leaf extract and phenolics, despite their antioxidant properties, promote nuclear accumulation of ChAP1. To study this dual role of ChAP1 we identified genes expressed in response to phenolics. Intradiol dioxygenase *CCHD1* is rapidly upregulated, independent of ChAP1 [2]. Coumaric acid caused rapid and simultaneous upregulation of most of the b-ketoadipate pathway genes. Deletion of *CCHD1* provided genetic evidence that protocatechuic acid is an intermediate in catabolism of many aromatics [3]. The activity of a structure series showed complementary requirements for upregulation of *CCHD1* and ChAP1 nuclear retention. The ability to metabolize a compound and ChAP1 nuclear retention are inversely correlated. To find additional genes induced by phenolics, microarrays designed from the predicted coding sequences of the *C. heterostrophus* genome [4] were hybridized to probes made from RNA of cultures exposed to coumaric acid, or controls. Expression of about 90 genes from different pathways primarily for metabolism, for example, the b-ketoadipate, quinic acid and shikimic acid pathways, as well as transporters from different families was altered in response to coumaric acid. The ability to respond to phenolics and detoxify or metabolize them via the b-ketoadipate pathway confers an advantage to plant pathogens, and explains the presence of at least two response pathways detecting these compounds. [1] Lev et al. (2005) Eukaryot. Cell 4:443-454; [2] Shanmugam et al. (2010) Cell. Microbiol. 12:1421-1434; [3] Shalaby et al. (2012) MPMI 25: 931-940; [4] Ohm et al. (2012) PLoS Pathog 8: e1003037. Supported in part by the Israel Science Foundation. We thank Michal Levin and Itai Yanai for help with microarray hybridization.

580. Mode of Action of Chitosan: Antifungal and Gene Modulator from Natural Origin. Luis V. Lopez-Llorca. Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) Ramon Margalef. University of Alicante, E-03080 Alicante, Spain. email: lv.lopez@ua.es.

Chitin is an abundant, easily obtained and renewable natural polymer, second only to cellulose. Chitin is a main structural component of barriers (cuticles and cell walls) of invertebrates (crustaceans, insects and nematodes) and fungi. Its deacetylated form, chitosan, has higher solubility and is known to have interesting biological properties. Chitosan, as a polycation, permeabilises the fungal membrane in an energy dependent manner. Chitosan kills or compromises the growth of important plant and human fungal pathogens. Unlike these fungi, fungal parasites of invertebrates (FPI, mainly nematophagous and entomopathogenic fungi), widely used biological control agents in sustainable agriculture, are resistant to chitosan. Perhaps as a result of coevolution with their hosts, FPI have evolved chitosan-resistant low-fluidity membranes (high content of saturated FFA) and produce efficient chitosan degrading enzymes. Besides, chitosan activates fungus development (e.g. conidiation) and expression of FPI pathogenicity factors such as serine proteases involved in the degradation of host barriers. Using chemogenomic platforms with yeast (sensitive to chitosan) we have identified chitosan putative gene targets. One of them, ARL1, a member of the Ras superfamily that regulates membrane trafficking, confers chitosan sensitivity as a deletion mutant and resistance when overexpressed in yeast. Yeast ARL1 overexpression in the presence of chitosan mainly caused down-regulation of genes involved in cell energy generation (mitochondrial biology, ATP metabolism, energy storage metabolites) and associated by-products (oxidative stress, ROS) and up-regulation of cell cycle progression (mitosis/meiosis, chromatin dynamics and sporulation) genes. *Neurospora crassa* conidia germination is particularly sensitive to chitosan. Low nutrient content of media increases chitosan driven membrane permeabilisation and *N. crassa* sensitivity. Using RNAseq we have found differential expression of genes involved in membrane permeability, cell energy/ROS generation and cell division as a response of *N. crassa* conidia to chitosan. Concluding, we are using cell and molecular approaches to fully understand the multimodal action of chitosan to fully exploit it in biotechnological and health applications.

581. Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains. Charissa de Bekker, David Hughes. Biology and Entomology, Center for Infectious Disease Dynamics,Pennsylvania State University, State College, PA.

Fungal entomopathogens rely on cellular heterogeneity during the different stages of insect host infection. Their pathogenicity is exhibited through the secretion of secondary metabolites. Infection strategies of this group of environmentally important fungi can thus be studied by analyzing their metabolome. Next to generalists such as *Beauveria bassiana* and *Metarhizium anisopliae*, specialist species exist that are able to control host behavior. One of the most dramatic examples is the death grip of ants infected by *Ophiocordyceps unilateralis*, where ants are being used as a vehicle and finally bite into vegetation before dying, aiding fungal spore dispersal after death. To establish this the fungus must not only overcome the immune system of the host, but also manipulate the brain and atrophy the muscles. To date, most work on manipulation of host behavior has described the ant's behavior,

leaving the molecular processes from the fungal point of view unresolved. To start unraveling the mechanisms underlying this phenomenon we are combining metabolite profiling with an *ex vivo* insect tissue culturing system that allows us to study fungal metabolites secreted in different parts within the host. Using this technique we established that *B. bassiana* and *M. anisopliae*, and *O. unilateralis* heterogeneously react to brain and muscle tissue by secreting a significantly different array of metabolites. The combination of these approaches with a concrete understanding of the host-parasite interaction in nature is allowing us to understand both the diversity of secondary metabolites as well as make discoveries regarding the temporal dynamics these fungi employ when releasing metabolites that affect the host. *This project is financed by the Marie Curie International Outgoing Fellowships and Penn State University* .

582. Gene expression of fungal aldehyde dehydrogenases in ectomycorrhiza. Catarina Henke[1,2], Kartrin Krause[1], Erika Kothe[1]. 1) Friedrich Schiller University of Jena, Institute of Microbiology, Microbial Communication, Neugasse 25, D-07743 Jena, Germany; 2) Max Planck Institute for Chemical Ecology, International Max Planck Research School, Hans-Knöll-Strabe 8, D-07745 Jena, Germany, chenke@ice.mpg.

Ectomycorrhizal fungi form a mutual symbiosis with trees and perform important functions in the ecosystem, particularly improving plant growth, nutrient supply and plant protection against pathogens. The molecular level of the association between the basidiomycete fungus *Tricholoma vaccinum* and the specific host spruce (*Picea abies*) is studied to investigate the molecular mechanisms of interaction. Differential display analysis revealed a fungal aldehyde dehydrogenase encoding gene *ald1* from the basidiomycete *T. vaccinum* specifically expressed in ectomycorrhiza during interaction with the compatible host. Ald1 has a key function in the detoxification of alcohols and aldehydes occurring in mycorrhizal biotopes and is involved in production of the phytohormone indole-3-acetic acid. Gene regulation of *ald1* is monitored *via* quantitative RT-PCR analyses. Transcription level was increased in the mutualistic association and could be stimulated by different external supplements, namely alcohols and aldehydes. The *ald1* overexpressing mutants generated by *Agrobacterium tumefaciens* mediated transformation showed increased ethanol stress tolerance. Linkage between gene transcription level and phenotypic characterization will elucidate deeper understanding of biological function, particularly of the possible pathways of auxin synthesis, and will allow better understanding of aldehyde dehydrogenases in ectomycorrhiza.

583. Interaction of ectomycorrhizal fungi with environment. Katrin Krause, Ines Schlunk, Erika Kothe. Institute of Microbiology, Friedrich Schiller University, Jena, Thuringia, Germany.

Ectomycorrhizal fungi play an important role in the biogeochemical cycle, act as decomposers and environmental indicators. Therefore they interact with different compounds of the environment like secondary metabolites of plant and soil living microorganisms, heavy metals and xenobiotics. Different enzymes and transporters are involved in these processes. One of these transporters, multidrug and toxic compound extrusion (MATE) *mte1* of the ectomycorrhizal fungus *Tricholoma vaccinum*, was upregulated during symbiosis. By heterologous expression of *mte1* in *Saccharomyces cerevisiae*, different metals, xenobiotics and secondary metabolites were identified as substrates for the MATE transporter. Furthermore, a retrotransposon *retro* showed an upregulation during the symbiosis with spruce. After long-term cultivation of the fungus an additional copy of the retrotransposon was detectable by Southern blot analyses in the fungal genome, showing transposition during co-cultivation with the plant. Since the fungus is prone to experience some plant-derived defense, the induction of transposition might be caused by plant induced stress.

584. Genetic exchange in an arbuscular mycorrhizal fungus; *Rhisophagus irregularis*. Pawel Rosikiewicz[1], Ian Sanders[2]. 1) Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland Pawel.Rosikiewicz@unil.ch; 2) Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland, Ian.Sanders@unil.ch.

Rhizophagus irregularis is a model species of an arbuscular mycorrhizal fungi (AMF). The AMF forms symbiotic relationship with roots of land plants, improving plant growth and protecting plants against parasites. *R. irregularis* is a particularly important species of AMF because it colonizes roots of most of crop plants such as rice, potato and wheat. However, different isolates of this fungus can affect plant phenotype differently. Moreover, it recently has been shown that two isolates of AMF can exchange genetic material, a process that can alter both, plant and fungal phenotypes. *R. irregularis*, is a coenocytic organism, which means that many nuclei coexist and can move in the common cytoplasm. The genetic exchange between two AMF isolates occurs via vegetative hyphal fusion. However, unlike in most fungi AMF produces multinucleate spores and it has been shown that each isolate of *R. irregularis* carries genetically different nuclei, which are maintained in successive AMF generations. What is unknown is the fate of parental nuclei after the genetic exchange, how many parental nuclei are exchanged and whether the mix of nuclei is random. In addition the nuclei are exchange with the surrounding cytoplasm. This lead to a question whether mitochondria from both parental isolates are transmitted to the offspring. In order to answer those questions I performed an *in vitro* experiment, where 6 isolates of *R. irregularis* were grown in pairs and allowed to fuse and exchange their cytoplasm. Subsequently, spores from all *in vitro* cultures were collected and used to establish 215 potentially crossed AMF lines. Each AMF line was established from a single spore. Fifty-seven of this newly produced AMF lines where genotyped, resulting in identification of 40 crossed AMF lines. All genotyped single spore lines carried only one mitochondrial haplotype. Moreover, all the progeny of a given pair of parental AMF isolates received the mitochondria from the same parent.

585. A surface hydrophobin in ectomycvorrhiza interaction. Dominik Senftleben, Katrin Krause, Erika Kothe. Friedrich-Schiller-Universitat, Jena, Germany.

Hydrophobins are small-secreted proteins with low sequence homology. However, all proteins contain eight cysteines, which form disulfide bridges. There are two classes of hydrophobins, depending on their solubility, which have a broad range of functions such as cell wall integrity, covering conidiospores, adhesion in pathogenic and ectomycorrhiza interactions. Therefore, hydrophobins as well as other genes in a mutual symbiosis are differential expressed. We showed this for hydrophobin *tthyd1*, which is up regulated in the Hartig'net during interaction of *Tricholoma terreum* with pine. We investigate hydrophobins in *T. vaccinum*, a widely spread basidiomycete (Agaricales - Tricholomataceae) which forms ectomycorrhiza with spruce. The establishment of a high compatible mycorrhiza needs in a co-culture system about one month, in comparison to a low compatible one, which needs about four months. We know which hydrophobin *T. terreum* regulates specifically in both interactions. Is this also the case for *T. vaccinum* and can we improve the low compatible interaction by heterologous or over expression? So far, we investigated five *T. vaccinum* hydrophobins and 17 in the Tricholoma genus in total. We also want to show *via* quantitative Real-Time PCR in which stage of the life cycle respectively symbiotic interaction *T. vaccinum* produces hydrophobins, what kind of role they play with respect to function in the symbiotic tissue and their regulation under heavy metal stress. Due to the fact that *T. vaccinum* is a k-strategist and the triggers for fruiting body development are unknown, over expression of hydrophobin protein are important to characterise their properties. Also *in silico* analyses are done such as protein-protein complexes with HADDOCK (High Ambiguity Driven protein-protein DOCKing) for rodlet layer formation and phylogenetic trees (ITS, hydrophobin) to understand how the evolution of the host, fungus and its hydrophobins took place. (Identification of a hydrophobin gene that is developmentally regulated in the ectomycorrhizal fungus *Tricholoma terreum*., Manke et al., App. and Environ. Microbiol., 2002 HADDOCK: a protein-protein docking approach based on biochemical and/or biophysical information. Bonvin et al., J. Am.

Chem. Soc, 2003).

586. Response of Alternaria brassicicola to the antifungal activity of isothiocyanates. Benoit Calmes, Jérôme Dumur, Thomas Guillemette, Nelly Bataillé-simoneau, Phillippe Simoneau. Institut de Recherche en Horticulture et Semences UMR1345, Angers, France.

Alternaria brassicicola is the causative agent of Brassicaceae black spot disease. This necrotrophic fungus causes glucosinolates degradation by plant myrosinase during infection. Isothiocyanates (ITCs), the major breakdown compounds, have been shown to be exert some toxicity to various *A. brassicicola* isolates. We showed, by application of specific fluorescent probes, that ITCs cause ROS production, disrupt the mitochondrial membrane potential and trigger apoptosis in *A. brassicicola* cells. The generation of an oxidative stress following ITCs application was confirmed by the fact that KO mutants deficient for the transcription factors AP1 and SKN7 (both being involved in the oxidative-stress response) are hypersensitive to ITCs and have decreased aggressiveness on glucosinolates accumulating host plants. Despite this host defense system, *A. brassicicola* is still able to complete its infectious cycle, indicating the existence of strategies to cope with this oxidative stress. Hypersensitivity to Al-ITC, Bz-ITC or Ph-ITC was observed in KO mutants deficient for distinct glutathione-S-transferases. Some of them were differentially expressed following exposure to ITCs and exhibited high transferase activity with ITCs as substrate. The polyol mannitol has been proposed to act as an antioxidant agent and protect fungal cells by quenching ROS produced by hosts in response to attack. We isolated the genes encoding the MPD and MDH enzymes, two essential enzymes of the mannitol metabolism in *A. brassicicola*, and used targeted gene disruption to create single and double mutants for each gene. Only mutants unable to accumulate mannitol in hyphae and conidia were sensitive to ITCs. Our results supported the involvement of fungal GST and mannitol metabolism in ITC-derived oxidative stress. GST participates in ITCs detoxification mechanisms and mannitol accumulation in protection against ROS. They highlight their importance with respect to the ability of *A. brassicicola* to efficiently accomplish its pathogen life cycle despite exposure to plant-derived antifungal metabolites.

587. Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont *Epichloë festucae*. Gemma M. Cartwright, Barry Scott, Yvonne Becker. Molec Biosci, Massey Univ, Palmerston Nth, New Zealand.

Reactive oxygen species (ROS) are emerging as important regulators required for the successful establishment and maintenance of the mutualistic association between the fungal endophyte *Epichloë festucae* and its grass host *Lolium perenne*. The generation of reactive oxygen species (ROS) by the fungal NADPH oxidase, NoxA has previously been shown to regulate hyphal growth of *E. festucae* in planta; a result that has led to the hypothesis that fungal-produced ROS are key second messengers in the symbiosis. However, the highly reactive nature of these molecules dictates that cells possess efficient sensing mechanisms to maintain ROS homeostasis and prevent oxidative damage to cellular components. The *Saccharomyces cerevisiae* Gpx3-Yap1 and *Schizosaccharomyces pombe* Tpx1-Pap1, two-component H_2O_2 sensors, serve as model redox relays for coordinating the cellular response to ROS. While proteins related to the Yap1 and Pap1 basic-leucine zipper (bZIP) transcription factors have been identified in a number of filamentous fungi, the components involved in the upstream regulation remain unclear. This study investigated the role of the *E. festucae* Yap1 homologue, YapA, and putative upstream activators GpxC and TpxA, homologues of Gpx3 and Tpx1, respectively, in responding to ROS. YapA is involved in responding to ROS generated at the wound site following inoculation into ryegrass seedlings. However, deletion of *yapA* did not impair host colonization indicating redundancy in systems used by *E. festucae* to sense and respond to plant-produced ROS. In culture, deletion of *E. festucae yapA*, renders the mutants sensitive to only a subset of ROS and this sensitivity is influenced by the stage of fungal development. In contrast to the H_2O_2-sensitive phenotype widely reported for fungi lacking the Yap1-like protein, the *E. festucae yapA* mutant maintains wild-type mycelial resistance to H_2O_2 but conidia of the yapA mutant are very sensitive to H_2O_2. Using a degron-tagged GFP-CL1 as a reporter, we found YapA is required for the expression of the spore specific catalase, *catA*. Moreover, YapA is activated by H_2O_2 independently of both GpxC and TpxA, suggesting a novel mechanism of regulation exists in *E. festucae*. This work provides a comprehensive analysis of the role and regulation of the AP-1 transcription factor pathway in a filamentous fungal species.

588. Genomic approaches to understand pathogenesis in the basidiomycete pathogen of food and energy crops, *Rhizoctonia solani*. Jonathan P. Anderson[1,2], James K. Hane[1], Rhonda Foley[1], Cynthia Gleason[1], Karam B. Singh[1,2]. 1) Plant Industry, CSIRO, Floreat, West Australia, Australia; 2) The UWA Institute of Agriculture, The University Of Western Australia, Crawley, West Australia.

Rhizoctonia solani is a broad-host-range necrotising fungal pathogen that is responsible for significant diseases to diverse crops. In Australia, *R. solani* most notably causes bare patch of cereals and costs $77 million pa in direct losses, while internationally it is a significant problem for global rice production. In the absence of strong host resistance, an understanding of fungus pathogenesis underpins alternative approaches to enhance resistance in crop plants. While the majority of phytopathogens sequenced to date belong to the Ascomycotina, *R. solani* is a basidiomycete with the closest sequenced relatives being biotrophic rust and smut fungi and saprophytic mushrooms, each possessing a lifestyle vastly different from *R. solani*. We have over-come complications associated with the multinucleate, heterokaryotic nature of *R. solani* to assemble a high quality consensus haploid genome of an AG8 isolate. Transcriptomics assisted with genome annotation and identified putative pathogenesis genes. Several of these genes display host-specific expression, while others show consistent infection-related expression across different anastomosis groups on different hosts. LC-MS based proteogenomics identified proteins from three fractions; soluble hyphal proteins, membrane localised proteins and secreted proteins, from *R. solani* growing in-vitro or in wheat infection mimicking conditions. QPCR confirmed up-regulation of some of the corresponding genes in infected wheat roots compared to *R. solani* grown in vitro, providing further support for a pathogenesis-related role. Functional testing of the role of candidate pathogenesis genes is on-going. On the plant side of the interaction, large scale gene expression and mutant analyses revealed the high degree of resistance in Arabidopsis (unlike its' susceptible relative, canola) was dependent on reactive oxygen species and not jasmonic acid, ethylene or salicylic acid. By contrast, moderate resistance in *Medicago truncatula* was dependent on ethylene mediated defences, which when over-activated, lead to enhanced resistance. These findings suggest that different plant species employ different defences with differing effectiveness against the same pathogen and a collective understanding of the interplay of host and fungal responses may facilitate novel strategies for enhancing resistance.

589. The Cpc1 (CpcA/Gcn4) regulator of the cross-pathway control of amino acid biosynthesis is required for plant infection of the vascular pathogen *Verticillium longisporum*. Susanna A. Braus-Stromeyer, Christian Timpner, Van Tuan Tran, Gerhard H Braus. Molecular Microbiology and Genetics, Georg-August-University, Goettingen, Germany.

The plant pathogenic fungus *Verticillium longisporum* is the causal agent of early senescence and ripening in *Brassica napus* (oilseed rape, Canola) and other cruciferous crops. *Verticillium* wilts have become serious agricultural threats during the last decades. *Verticillia* infect host-plants through the roots and colonize xylem vessels of the host-plant, which provide an environment with limited carbon sources. *V. longisporum* induces the cross-pathway control in the xylem fluid to cope with an imbalanced amino acid supply.

The transcriptional activator gene *VlCPC1* (similar to CpcA/GCN4) was knock-downed via RNA-mediated gene silencing and the expression of the two *CPC1* isogenes (*VlCPC1-1*, *VlCPC1-2*) in *V. longisporum* could be reduced up to 85%. The resulting mutants were more sensitive to amino acid starvation induced by 5-methyltryptophane (5-MT). In plant infection assays, the silenced mutant showed significantly less symptoms such as stunting and early senescence. Knockouts of *CPC1* in the haploid *V. dahliae* were sensitive to amino acid starvation and strongly reduced in symptom formation in their host *Solanum lycopersicum* (tomato).

The hybrid *V. longisporum* and the haploid *V. dahliae* are the first phytopathogenic fungi, which were shown to require *CPC1* for infection and colonization of their respective host plants oilseed rape and tomato.

590. Fungal-Specific Transcription Factor *AbPf2* Activates Pathogenicity in *Alternaria brassicicola* . Yangrae Cho[1], Robin Ohm[2], Igor Grigoriev[2], Akhil Srivastava[1]. 1) Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI; 2) United States Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598.

Alternaria brassicicola is a successful saprophyte and necrotrophic plant pathogen. Molecular determinants of its life style shift between saprophyte and pathogen, however, are unknown. To identify these determinants we studied nonpathogenic mutants of a transcription factor-coding gene, *AbPf2*. Frequency and timing of germination and appressorium formation on host plants were similar between the nonpathogenic D*abpf2* mutants and wild-type *A. brassicicola*. The mutants were also similar in vitro to wild-type *A. brassicicola* in vegetative growth, conidium production, and responses to chemical stressors, such as a phytoalexin, reactive oxygen species, and osmolites. The mutants, however, did not penetrate host plant tissues, though their hyphae continued to grow on the plant surface. Transcripts of the *AbPf2* gene increased exponentially soon after wild-type conidia encountered their host plants. A small amount of AbPf2 protein, monitored by fused green fluorescent protein, was located in both the cytoplasm and nuclei of young, mature conidia. The protein level decreased during saprophytic growth but increased several-fold during pathogenesis. Levels of both the proteins and transcripts sharply declined following colonization of host tissues beyond the initial infection site. When the transcription factor was expressed at an induced level in the wild type during early pathogenesis, the expression of 106 fungal genes was down-regulated in the D*abpf2* mutants. Notably, 33 of the 106 genes encoded secreted proteins, including eight putative effector proteins. Plants inoculated with D*abpf2* mutants expressed higher levels of genes associated with photosynthesis, the pentose phosphate pathway, and primary metabolism, but lower levels of defense-related genes. Our results suggest that conidia of *A. brassicicola* are programmed as saprophytes, but become parasites upon contact with their hosts. AbPf2 coordinates this transformation by expressing pathogenesis-associated genes, including those coding for effectors.

591. WITHDRAWN

592. Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus. Weiguo Fang, Xiaoxuan Chen. College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China.

Inspite being of great concern to human health and the management of plants and animals, the mechanisms facilitating host switching of eukaryotic pathogens remain largely unknown. The endophytic insect-pathogenic fungus Metarhizium robertsii evolved directly from endophytes and its entomopathogenicity is an evolutionarily acquired characteristic. We found that M.robertsii acquired a sterol carrier (Mr-NPC2a) from an insect by horizontal gene transfer (HGT). Mr-NPC2a increased the amount of ergosterol in hyphal bodies by capturing sterol from insect hemolymph, and thus maintained cell membrane integrity and improved fungal survival rate. On the other hand, the reduction in sterol (substrate for molting hormone synthesis) in insect hemolymph elongated larval stage, which allows the fungus to fully exploit host tissues and produce more conidia. This is first report of HGT from host to a eukaryotic pathogen, and the host gene ultimately improved the infectivity of the pathogen.

593. Characterization of the *CoPRF1* mutant of *Colletotrichum orbiculare* defective in evasion of host defense responses. Kaoru Tanaka, Yasuyuki Kubo. Graduate School of Life and Environmenrtal Sciences, Kyoto Prefectural University, Kyoto, Japan.

Plant pathogens have co-evolved with their host plants which have evolved the defense system against their pathogens. It is generally accepted that plants express basal immunity by the recognition of the pathogen-associated molecular patterns, but compatible pathogens suppress the plant basal defense by secreting effector proteins. In our previous study, we have obtained several pathogenicity deficient insertional mutants in *Colletotrichum orbiculare* by *Agrobacterium tumefaciens*-mediated transformation (AtMT). Among them, in the mutant named YK4524 it was shown that a T-DNA insertion disrupted a gene which presumably encodes an extracellular protein with signal peptide sequence. And BLAST search of the predicted sequence found no significant homologous genes in published databases, suggesting that it is unique to *C. orbiculare*. So we named this gene *CoPRF1* (Pathogenesis-related factor). Target gene disruption mutants of *coprf1* obtained by AtMT showed significant reduction in virulence on the host leaves. However, characteristics such as germination, appressorium formation and penetration hyphal formation of *coprf1* mutants *in vitro* were normal, indicating that *CoPRF1* is not essential for infection related morphogenesis. On the other hand, penetration ability of mutants was attenuated on intact cucumber cotyledons, and the elongation of its invasive hyphae was slower compared with the wild type. To confirm the possibility that decreased virulence of coprf1 mutants was involved in plant defense responses, we inoculated *coprf1* mutant on cucumber cotyledons of which defense responses was disturbed by transient heat-shock. As expected, the pathogenicity of *coprf1* mutant was restored. Furthermore, expression analysis of *CoPRF1* by RT-PCR showed that *CoPRF1* expressed *in planta*, but not *in vitro* culture. From these results, it was suggested that *CoPRF1* would engage in evasion of host basal resistance at the host infection.

594. *CPS1* mutants in *Coccidioides* are avirulent and act as an attenuated vaccine in the valley fever mouse model. Hema P. Narra[1,4], Lisa F. Shubitz[2,3], M. Alejandra Mandel[1,3], Leslie Gunatilaka[5], Hien Trinh[2,3], Marc J. Orbach[1,3]. 1) School of Plant Sciences, Univ. of Arizona, Tucson, AZ; 2) Veterinary Sciences and Microbiology, Univ. of Arizona, Tucson, AZ; 3) Valley Fever Center for Excellence, Univ. of Arizona, Tucson, AZ; 4) Department of Pathology, Univ. of Texas, Medical Branch, Galveston, TX; 5) School of Natural Resources and the Environment, Univ. of Arizona, Tucson, AZ.

Coccidioides species are mammalian pathogens endemic to the Southwestern US as well as parts of Mexico, Central and South America. The disease they cause, coccidioidomycosis, or valley fever, is considered an emerging infectious disease due to increases in reported cases over the past 10 years. To identify virulence factors of this pathogen that may be targets for therapeutics, we have identified and disrupted genes that are important for pathogenicity in both plant pathogens and other animal pathogens. Based on the work of Liu et al. (2003), we disrupted the *Coccidioides* ortholog of *C. heterostrophus CPS1* in *C. posadasii* strain Silveira. *CPS1* was originally identified as a potential non-ribosomal peptide synthase component, because it encodes a polypeptide with two AMP binding domains related to the adenylation domains in bacterial non-ribosomal peptide synthases. However it also contains a putative N-terminal DMAP1b domain. In mammals, this domain binds the DMAP1 transcriptional co-repressor that has been shown to bind regulatory proteins and is proposed to act as a co-repressor of transcription. The *C. posadasii cps1* deletion strain is non-pathogenic in susceptible mice but does initiate the formation of spherules, the infectious form of *Coccidioides*. The mutant also forms spherules *in vitro*. Whether Cps1 plays a role as a regulator of virulence via the DMAP1b domain, or via production of a potential toxin is not known. This is being explored via RNA-seq analysis and isolation of secreted metabolites from both the wild type strain Silveira and the *cps1* mutant. The *cps1* mutant appears to have great potential as an attenuated vaccine since it protects from infection; when susceptible mice are challenged with wild type *C. posadasii* after inoculation with the *cps1* mutant, nearly all experience extended survival of at least four weeks and have low fungal burdens. inoculation with the *cps1* mutant, they are completely resistant to infection. Lu, S. W., S. Kroken, B. N. Lee, B. Robbertse, A. C. L. Churchill, O. C. Yoder, and B. G. Turgeon. 2003. A novel class of gene controlling virulence in plant pathogenic ascomycete fungi. Proceedings of the National Academy of Sciences of the United States of America 100:5980-5985.

595. Elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors. Lauren A. Du Fall, Peter S. Solomon. Research School of Biology, Australian National University, Canberra, ACT, Australia.

The dothideomycete *Stagonospora nodorum* is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB). This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effector proteins that are internalized into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a multi-omics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To further understand the host response to SnToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate SnToxA causes an increase in defence-related secondary metabolites. The effect of these metabolites on *Stagonospora nodorum* growth and sporulation *in vitro* and *in planta* has identified several compounds with novel anti-fungal properties. These complementary approaches have provided a novel insight into the contribution of the SnToxA effector protein to SNB in wheat.

596. Nep1-like proteins of the downy mildew *Hyaloperonospora arabidopsidis* trigger immunity, but not necrosis, in the Arabidopsis host. Stan Oome[1,2], Adriana Cabral[1], Simon Samwel[1], Tom Raaymakers[1], Guido Van den Ackerveken[1,2]. 1) Plant-Microbe Interactions, Utrecht University, Utrecht, Netherlands; 2) Centre for BioSystems Genomics (CBSG), Wageningen, Netherlands.

The genome of the downy mildew pathogen *Hyaloperonospora arabidopsidis*, an obligate biotrophic oomycete, encodes several necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP). The *NLPs* of *H. arabidopsidis* (*HaNLPs*) constitute a family of 12 genes and 15 pseudogenes, most of which form a species-specific clade separate from NLPs of related *Phytophthora* species, suggesting that the family has recently expanded. The secreted HaNLPs were found to be nontoxic when tested on Arabidopsis or tobacco, in contrast to known necrosis-inducing NLPs, e.g. the *P. sojae* PsojNIP protein that is cytolytic and induces a strong cell death response in dicot plant tissues. Even HaNLP3, which is most similar to necrosis-inducing NLP proteins of other oomycetes, and which contains all amino acids that are known to be important for necrosis-inducing activity, did not induce necrosis. Chimeras constructed between HaNLP3 and the necrosis-inducing PsojNIP protein demonstrated that most of the HaNLP3 protein is functionally equivalent to PsojNIP, except for an exposed domain that prevents necrosis induction. The *HaNLP* genes are mostly expressed early during infection, suggesting an alternative function of noncytolytic NLP proteins during biotrophic infection of plants. To investigate if HaNLP production in the host affects susceptibility to infection, transgenic Arabidopsis plants were generated. Surprisingly, overexpression of HaNLP3, 5, 6, 9, and 10 resulted in plants with severely reduced growth. To be able to monitor NLP-effects on pathogen infection, in the absence of growth reduction, an Arabidopsis line with an estradiol-inducible *HaNLP3* construct was generated. DNA microarray analysis revealed that plant immune responses were strongly activated upon estradiol-induced *HaNLP3* expression. Furthermore, resistance to *H. arabidopsidis* infection was activated, suggesting that the plant is able to recognize the pathogen-associated

HaNLP3 protein and mount an effective immune response. Our research is now focused on determining how Arabidopsis is able to respond to the HaNLPs and how the downy mildew pathogen can suppress the host immune response triggered by non-toxic NLPs.

597. Genes important for *in vivo* survival of the human pathogen Penicillium marneffei. Harshini C. Weerasinghe, Michael J. Payne, Hayley E. Bugeja, Alex Andrianopoulos. Genetics, The University of Melbourne, Parkville, Victoria, Australia.

Pathogenic fungi are having an increasing global impact in the areas of health, agriculture and the environment. As such it is essential to understand the mechanisms that fungi employ to survive and grow within a host. The emergence of many new "opportunistic fungal pathogens" has to a great extent altered the traditional view that pathogenicity was solely reliant on the inherent properties of the pathogen. In fact, the ability of a pathogen to cause disease in some hosts but not in others suggests that pathogenic determinants are complex and dynamic, and are largely dependent on specific pathogen-host relationships. Despite this there are conserved aspects of the interactions between host and pathogen. For example., hosts employ innate immune responses as an almost immediate recognition and attack mechanism against invading pathogens. Penicillium marneffei is a temperature dependent dimorphic fungus, growing in a hyphal form producing conidia at 25°C and as a yeast form at 37°C. Despite its importance as an opportunistic pathogen, little is known about the biology and mechanism of infection of P. marneffei. The infectious agents (conidia) are believed to be inhaled, reaching the alveoli of the lungs, where they are phagocytosed by alveolar macrophages for elimination. At this point that P. marneffei switches growth to a pathogenic yeast cell form, and is able to withstand macrophage cytotoxic attacks to cause infection. In order to understand how P. marneffei responds to the host, RNA-seq analysis was used to create a transcriptomic profile of P. marneffei, when infected in murine macrophages. These results were compared to RNA-seq data from hyphal (25°C) and yeast (37°C) cells grown *in vitro* in order to identify genes that are specifically upregulated during infection. Based on this analysis a group of genes of varying functions were chosen for gene deletion studies and tested for defects in pathogenicity. Among these is a group of Pep1-like aspartic endopeptidases which are a uniquely expanded family in P. marneffei and that show reduced virulence in a macrophage model.

598. Oxalate-minus mutants of *Sclerotinia sclerotiorum* via T-DNA insertion accumulate fumarate in culture and retain pathogenicity on plants. Liangsheng Xu[1], Meichun Xiang[1], David White[1], Weidong Chen[1,2]. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Washington State University, Pullman, WA 99164.

Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen capable of infecting over 400 plant species including many economically important crops. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor for *S. sclerotiorum* through several mechanisms. During our random mutagenesis study of *S. sclerotiorum* using *Agrobacterium*-mediated transformation, we identified three mutants that had lost oxalate production. Southern hybridization blots showed the mutation was due to a single T-DNA insertion, and plasmid rescue and DNA sequencing confirmed that the T-DNA insertion site was located in the ORF of oxaloacetate acetylhydrolase (*Ssoah*, SS1G_08218) of *S. sclerotiorum*. The mutants did not change the color of a pH-indicating medium (PDA amended with 50 mg/L bromophenol blue). The pH values of 6-day PDB culture filtrates were 1.8-2.0 for the wild type and 2.8-3.1 for the mutants. No oxalic acid was detected using HPLC in culture filtrates or in the mycelium of the mutants, but another acid compound was accumulated in culture filtrates of the mutants and detected by HPLC, and the compound was identified as fumaric acid using LC-MS. The mutants showed reduced vegetative growth on PDA and produced sclerotia that are beige in color and soft in texture. Artificial acidic conditions (pH 3.4 and 4.2) enhanced vegetative growth and promoted normal (black and hard) sclerotial formation of the mutants. Furthermore, the oxalate-minus mutants retained pathogenicity on pea, green bean and faba bean in detached leaf assays and on intact plants of *Arabidopsis thaliana*, and their virulence levels were similar to that of the wild type strain on certain host plants, but varied depending on the plant species tested. The mutant had increased expression levels of cell wall-degrading enzymes such as polygalacturonases compared to the wild type strain during the process of infecting pea leaves. The results showed that a low pH condition is very important for growth and virulence of *S. sclerotiorum* on its wide range of host.

599. Molecular characterization of fungi associated with superficial blemishes of potato tubers in Al-Qasim region, Saudi Arabia. Rukaia M Gashgari[1], Youssuf A. Gherbawy[2]. 1) Biology Dept, King Abdulaziz university, Jeddah, Saudi Arabia; 2) Biology Dept, Taif university, Taif , Saudi Arabia.

Potato (Solanum tuberosum) becoming a more and more important foodstuff in the world. Also, the visual quality of fresh potatoes became a dominant criterion and a significative economical issue in potato market. According the vegetative reproduction of this species, requirements for visual quality are also needed for potato tubers. As an organ for reserve and propagation, the tuber grows underground and is in contact with soil-borne microorganisms, making it potentially exposed to blemishes. Some blemishes are due to known pathogens and others whose causes are unknown are called atypical blemishes. Therefore, knowledge about the pathogens is needed to set up efficient control strategies and to help potato growers to better know the causes of these blemishes and find technical solutions for improving the potato quality. Therefore, the objective of this proposed research study is the possibility of using some modern methods of molecular diagnostics and rapid detection of the presence of fungal contaminants in potato blemishes in Al-Qasim (Saudi Arabia). Polygonal lesions was the most observed blemish type in the collected samples. One hundred and sixty isolates were collected from different types of blemishes recorded in this study. Fusarium , Penicillium, Ilyonectria, Alternaria and Rhizoctonia were the most common genera collected from different blemish types. Using ITS region sequencing all collected fungi identified the species level. All Fusarium strains colled during this study were use to detect its pathogenicity against potato tubers. The inoculated fungi were re-isolated from the diseased potato tubers to prove the Koch's postulates. This is the first comprehensive report on identity of major pathogenic fungi causing potato dry rot isolated from potato tuber blemishes in Saudi Arabia.

600. Patterns of Distribution of Bacterial Endosymbionts in Lower Fungi. Olga Lastovetsky[1], Xiaotian Qin[2], Stephen Mondo[2], Teresa Pawlowska[2], Andrii Gryganskyi[3]. 1) Microbiology Dept, Cornell University, Ithaca, NY; 2) Plant Pathology & Plant-Microbe Biology Dept, Cornell University, Ithaca, NY; 3) Biology Dept, Duke University, Durham, NC.

Fungi are not typically known to have endosymbionts. However, some members of Glomeromycota and Mucoromycotina have recently been found to harbor bacteria in their hyphae and spores. The newly discovered association between *Rhizopus microsporus* (Mucoromycotina) and *Burkholderia* bacteria (betaproteobacteria) prompted us to search for endobacteria in other members of Mucoromycotina fungi. We screened a broad range of Mucoromycotina isolates for the presence of bacterial endosymbionts using PCR with universal and *Burkholderia*-specific primers that targeted the 16S and 23S rRNA bacterial genes. Endobacteria were only found in certain strains of *R. microsporus* but in no other *Rhizopus* or Mucoromycotina isolates. A 28S rRNA gene phylogeny of the screened fungal isolates revealed a clustering of bacteria(+) *R. microsporus* isolates away from bacteria(-) *R. microsporus* isolates. To explore this putative divergence within the *R. microsporus* lineage we are working on a multi-gene phylogeny of *Rhizopus* isolates, which is based on multiple coding and non-coding regions.

601. Phylogenetic and genomic analysis of a novel, nematophagous species of *Brachyphoris*. S. Sharma Khatiwada, J. B. Ridenour, A. Thomas, J. Tipton, T. Kirkpatrick, B. H. Bluhm. University of Arkansas, Fayetteville, AR.

Plant-parasitic nematodes are destructive pathogens of crops worldwide. The phase out of many chemical control methods has prompted a search for feasible alternative control strategies. Nematophagous fungi are widely distributed in terrestrial and aquatic environments, and have evolved diverse strategies to parasitize nematodes. In this study, a previously characterized but unnamed nematophagous fungus (designated TN14) was taxonomically classified and a draft genome sequence was obtained. Taxonomic identification of the fungus was conducted using the ITS1-5.8S-ITS2 rDNA sequences. Phylogenetic relationships were inferred with neighbor-joining and maximum likelihood methods. Based on the primary GenBank database search, the ITS region of TN14 was compared with the ITS region of 41 taxa. From this analysis, the fungus is predicted to form a distinct monophylogenetic clade with *Brachyphoris*, a genus of nematophagous fungi related to *Dactylella* and *Vermispora*. Although some 200 species of nematophagous fungi are known, publicly available resources are very limited. Thus, we obtained a draft sequence of the TN14 genome via Roche-454 sequencing technology. Alignment of over 90% of the sequenced reads revealed an estimated genome size of 100.1 MB, which is notably larger than the genomes of many other ascomycetes, including that of the only other sequenced nematophagous fungus, *Arthrobotrys oligospora* (40.07 Mb). Subsequent analyses of the genome of TN14 are providing insight into molecular mechanisms underlying pathogenicity and the viability of TN14 as a potential bio-control agent in agricultural settings.

602. The proteome of the traps of the nematode-trapping fungus *Monacrosporium haptotylum* . K-M. Andersson[1], T. Meerupati[1], F. Levander[2], E. Friman[1], D. Ahrén[1], A. Tunlid[1]. 1) Microbial Ecology, Department of Biology, Lund University, Sweden; 2) Protein Technology, Department of Immunotechnology, Lund University, Sweden.

Nematode-trapping fungi have for a long time been seen as putative biological control agents against parasitic nematodes. A better knowledge on the infection process will facilitate the development of these fungi as biological control agents and may also lead to the discovery of new nematicidal drugs. *Monacrosporium haptotylum* is a nematode-trapping fungus that captures nematodes using an adhesive trap called knob. In this study, proteins were extracted from knobs and mycelium and analyzed using SDS-PAGE combined with LC/MS/MS. Peptides were matched against predicted gene models from the recently sequenced genome of *M. haptotylum*. Furthermore, the transcriptome in the knob during infection of nematodes were analyzed.

The analysis showed that there was a large difference in the proteome of the knob compared to the mycelium. In total 336 proteins were identified. A quantitative analysis showed that 54 proteins were expressed at significantly higher levels in the knobs versus the mycelium. Proteins containing a predicted secretion signals were overrepresented in knobs (knobs 41 %; mycelium 11 %). Five of the secreted proteins upregulated in knob were small secreted proteins (SSPs). Three of the SSPs were orphans since they showed no homology to the NCBI database and lack pfam domains. Interestingly, two of them are upregulated in the transcriptome during infection of nematodes.

Among the upregulated proteins were several putative cell-surface adhesins containing the carbohydrate binding domain WSC and repetitive regions enriched in threonine/serine residues. Upregulated were also a diverse array of peptidases including serine endopeptidase (subtilisin), aspartic endopeptidase, metalloendopeptidase, aminopeptidase and carboxypeptidase. Several proteins related to stress response and basic metabolism were also identified in the trap proteome. During infection of nematodes, genes with the domains peptidase_S8 (subtilisin), DUF3129 and WSC are highly upregulated in the knob.

Taken together, our analysis shows that the trap cell has a unique proteome containing components that are involved in the early stages of infection including adhesion and penetration of the nematode.

603. Sequencing the *in planta* transcriptomes of *Colletotrichum* species provides new insights into hemibiotrophy. Richard J. O'Connell[1], Stéphane Hacquard[1], Jochen Kleemann[1], Emiel Ver Loren van Themaat[1], Stefan Amyotte[2], Michael Thon[3], Li-Jun Ma[4], Lisa Vaillancourt[2]. 1) Max Planck Institute for Plant Breeding Research, Cologne, Germany; 2) Department of Plant Pathology, University of Kentucky, Lexington, KY; 3) CIALE, Universidad de Salamanca, Villamayor, Spain; 4) Department of Biochemistry and Molecular Biology, UMASS Amherst, MA.

Colletotrichum species cause devastating diseases on crop plants worldwide. Infection involves formation of a series of specialized cell-types associated with penetration (appressoria), growth inside living host cells (biotrophic hyphae) and tissue destruction (necrotrophic hyphae). To analyse the transcriptional dynamics underlying these transitions, we used RNA sequencing to compare the transcriptomes of *C. higginsianum* infecting *Arabidopsis* and *C. graminicola* infecting maize. The early transcriptome is dominated by secondary metabolism and effector genes, suggesting both appressoria and biotrophic hyphae are platforms for delivering protein and small molecule effectors to host cells. Genes encoding a vast array of wall-degrading enzymes, proteases and membrane transporters are up-regulated at the switch to necrotrophy, when the pathogen mobilizes nutrients from dead cells for growth and sporulation. However, the two species employ different strategies to deconstruct plant cell walls that are adapted to their host preferences. Thus, *C. higginsianum* activates more pectin-degrading enzymes during necrotrophy, whereas *C. graminicola* mostly activates hemicellulases and cellulases at this stage. Remarkably, although appressoria formed *in vitro* are morphologically similar to those *in planta*, comparison of their transcriptomes showed >1,500 genes are induced only upon host contact, suggesting that sensing of plant signals by appressoria dramatically reprograms fungal gene expression in preparation for host invasion.

604. Biological activities of natural products synthesized by the mammalian fungal pathogen, Histoplasma capsulatum. A. Henderson[1], M. Donia[2], M. Fischbach[2], A. Sil[1]. 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA.

Histoplasma capsulatum is a soil fungus that infects healthy mammalian hosts upon inhalation. Extrapolating from previous work, we hypothesized that small-molecule natural products produced by Histoplasma are enriched for activity against host molecular targets. Using a bioinformatics approach, we identified biosynthetic gene clusters in strain G217B containing genes required for natural product synthesis in other organisms: nonribosomal peptide synthetases (NPS) and polyketide synthases (PKS). Experimentally, we found that partially purified compounds from Histoplasma culture supernatants are able to buffer supernatants against acidic challenge and promote macrophage lysis. Both activities are relevant to virulence in mammalian hosts. We are structurally characterizing the relevant natural products using preparative HPLC, MS and NMR. In a complementary approach, we used RNA interference to target the complete set of NPS and PKS genes identified in the Histoplasma genome. We are using the resultant mutant strains to correlate biosynthetic genes with small molecule production, and to assess the role of these genes in pathogenesis.

605. From antagonism to synergism: roles of natural phenazines in bacterial-fungal interactions between Pseudomonas aeruginosa and Aspergillus fumigatus. He Zheng[1], Fangyun Lim[2], Jaekuk Kim[1], Mathew Liew[1], John Yan[1], Neil Kelleher[1], Nancy Keller[2], Yun Wang[1]. 1) Northwestern University, Evanston, IL, USA; 2) University of Wisconsin-Madison, Madison, WI, USA.

Secreted small molecules are increasingly recognized to mediate many types of bacterial-fungal interactions in nature and the clinical environment,

which can have enormous impacts on human and ecosystem health. Despite their ubiquity and importance, very little is known about the molecular mechanisms underlying these interactions. To address this, we select to study the interactions between Pseudomonas aeruginosa and Aspergillus fumigatus, the ubiquitous opportunistic bacterial and fungal pathogens, respectively, via redox-active bacteria-secreted phenazines. We hypothesize that the functions of these molecules are multifactorial, dependent on genetic and environmental factors. By combining genetic, physiological, electrochemical, and metabolic profiling strategies, here we report that redox-active phenazines can mediate biofilm interactions between P. aeruginosa and A. fumigatus in multiple ways, ranging from antagonistic to synergistic. We find that phenazine production patterns are generally correlated with bacterial-fungal interaction phenotypes, in a genetically- and temporarily-dependent manner. Further, fungi can convert the precursor phenazine-1-carboxylate (PCA) produced by bacteria into several other phenazines. These structurally related phenazines come in with characteristic physical-chemical properties including redox properties. Our most striking finding is to be able to draw connections between a phenazine's structure and its mode of action. Under one given condition, some phenazines such as phenazine-1-carboxamide (PCN) can facilitate bacterial biofilm development by inhibiting fungal development; some others such as pyocyanin (PYO) show no apparent effect on fungal development; and 5-methyl-phenazine-1-carboxylic acid (5-Me-PCA) can synergistically facilitate both bacterial and fungal biofilm developments. In addition, we find that changing the ambient redox and pH conditions can affect a phenazine's mode of action, likely via influencing its redox activity. Taken together, our findings imply that phenazines-mediated bacterial-fungal interactions have profound and diverse effects on multicellular behavior in competitive and mixed-species biofilm environments.

606. Genomic analysis of Mortierella elongata and its endosymbiotic bacterium. Gregory Bonito[1], Andrii Gryganskyi[1], Christopher Schadt[2], Dale Pelletier[2], Amy Schaefer[3], Gerald Tuskan[2], Jessy Labbé[2], Sofia Robb[4], Rebecca Ortega[1], Francis Martin[5], Mitchel Doktycz[2], Kurt LaButti[6], Matt Nolan[6], Robin Ohm[6], Igor Grigoriev[6], Rytas Vilgalys[1]. 1) Duke University, Durham NC; 2) Oak Ridge National Laboratory, Oak Ridge TN; 3) University of Washington, Seattle WA; 4) University of California, Riverside CA; 5) Institut National de la Recherche Agronomique, Nancy France; 6) Joint Genome Institute, Walnut Creek CA.

Mortierella belong to a group of basal fungi (Mortierellomycotina) common to soils and the rhizosphere and endosphere of many plant species. Mortierella species are known for rapid growth and abundant lipid production. Mortierella elongata is one species commonly isolated from forest soils and healthy plant roots where it grows asymptomatically as an endosymbiont. Mortierella elongata is a heterothallic species but can also reproduce asexually through chlamydospores and sporangiospores. Recent reports indicate that some isolates of M. elongata host endosymbiotic bacteria, which may be transmitted vertically via spores. However, it is still unclear whether all Mortierella species host endosymbionts or whether these are lineage-specific associations. Given the geographically widespread distribution of Mortierella elongata and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont to address whether there are genomic signatures of co-adaptation or co-evolution in the genomes of Mortierella and its endosymbiotic bacterium, which may impact the function and growth of Mortierella elongata. The 50 Mb genome of M. elongata was sequenced to 112x coverage. Of the 220,113 putative proteins identified in M. elongata, 109,093 appear to be unique (e.g. only ~50% have orthologs in other fungal species having sequenced genomes). The M. elongata genome appears to be enriched in genes related to tryptophan metabolism, siderophore group nonribosomal peptides, glucan 1,4-alpha glucosidases, and in lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophopholids) compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the M. elongata isolate is related to Glomeribacter (endosymbiont of Gigospora, Scutellospora, and other Glomeromycota) within the Burkholdariales. The ~2.6 MB endosymbiont genome is larger than that of Glomeribacter but quite reduced compared to free-living isolates of Burkholdaria. The reduced genome size of this bacterium, and the fact that it has thus far evaded pure culture isolation, supports the view that this is an ancient and obligate symbiosis.

607. Diversity and Content of Maize Leaf Endophytes are Correlated With Maize Genotype. Alice C. L. Churchill[1*], Santiago X. Mideros[1], Peter Balint-Kurti[2], Surya Saha[1], Rebecca J. Nelson[1]. 1) Department of Plant Pathology & Plant-Microbe Biology, Cornell Univ, Ithaca, NY; 2) USDA-ARS Plant Science Research Institute, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

All plants contain endophytes that have the potential to provide fitness benefits to their hosts by increasing tolerance to environmental stressors, boosting plant nutrition and growth, and providing increased resistance or tolerance to insect pests and plant pathogens. We are characterizing endophytic populations inhabiting aboveground maize tissues with the goal of associating maize genetic variation with the diversity, structure and constitution of maize-associated microbial communities. Nine maize lines, representing a diverse subset of the founders of the NAM (Nested Association Mapping) population, were grown at a single North Carolina field site in 2012 and assayed for culturable endophytic bacteria and fungi. Two distinct seed sources for each maize line were planted in a randomized experimental design, and three replicates per seed source were assayed, representing a total of 54 samples. Leaf pieces were harvested just prior to pollination for each maize line, surface sterilized using standard endophyte isolation methodologies, and ground leaf extracts were cultured on four media that select for slow- and fast-growing fungi and copiotrophic, diazotrophic, and oligotrophic bacteria. Approximately 65% of the samples contained one or more phenotypically distinct, culturable bacteria, 28% contained one or more fungi, 22% contained both bacteria and fungi, and endophytes were undetectable in 28% of the samples. A greater number and diversity of fungi were cultured from tropical maize lines than from temperate lines. Bacteria were isolated from all maize lines, with some lines exhibiting significantly greater microbial community diversity than others. Several phenotypically similar bacteria and fungi were isolated from multiple maize lines. Microbial identity via 16S and ITS sequencing, as well as identification of unculturable endophytes via whole genome metagenomic sequencing, are in progress. We are particularly interested in identifying members of the microbiome that modulate disease symptoms caused by maize leaf and ear pathogens. Hence, future studies will focus on in vitro and in planta endophyte-pathogen interactions.

608. Characterisation of epichloae endophytes from the Triticeae and their potential use in modern cereals. Wayne R Simpson, Marty J Faville, Roger A Moraga, Richard D Johnson. Agresearch Grasslands, Palmerston North, New Zealand.

Epichloae endophytes infect grasses within the subfamily Pooideae including some within the tribe Triticeae. There have been no accounts of modern domesticated Triticeae hosting epichloae endophytes but there have been reports in *Elymus*, *Hordeum* and other grasses within the tribe. Our goal is to isolate epichloae endophytes from the wild relatives of modern cereals and inoculate these into modern cereal crops. We surveyed populations of *Elymus* and *Hordeum*, primarily from Asia, and selected 29 *Elymus* and 13 *Hordeum* infected plants. We used simple sequence repeats (SSR) and b-tubulin sequencing to determine genetic similarity, hybrid status and closest non-hybrid ancestor. SSR data indicates 26 genetically distinct strains that fall into 5 major clades. b-tubulin analysis shows that the majority of our isolates had *Epichloë bromicola* ancestry, with both hybrid and non-hybrid strains identified. Within the non-hybrid *E. bromicola* two major clades were identified. Of the hybrids we identified examples of *E. bromicola* x *E. typhina* and *E. bromicola* x *E. amarillans*. Although *E. bromicola* has been observed in Asian grasses we believe that this is the first report of an *E. bromicola* x *E amarillans* hybrid. Of the remaining isolates, we found examples of strains with *E. yangzii* ancestry (all non-hybrids) and *E. elymi* ancestry (both non-hybrid and

E.elymi x *E. amarillans*).

609. The Interaction of *Mycoplasma*-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host. Mizue Naito[1], Teresa Pawlowska[2]. 1) Dept. of Microbiology, Cornell University, Ithaca, NY; 2) Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY.

Arbuscular mycorrhizal fungi (AMF), comprising the monophyletic phylum Glomeromycota, are obligate biotrophs, and form symbiotic associations with 80% of terrestrial plants. AMF associate symbiotically with the roots of plants, and are specialized in the transfer of nutrients from the soil to the plant host. In return for increased nutrient uptake, the plants supply AMF with up to 20% of their photosynthetically derived carbohydrates. Thus, AMF symbiosis contributes significantly to global nutrient cycling and terrestrial ecosystems. AMF have been known to harbour two types of bacteria in their cytoplasm: (i) the *Burkholderia*-related *Candidatus* Glomeribacter gigasporarum and (ii) a *Mycoplasma*-related bacteria, which we refer to as *Mycoplasma*-related endobacteria (MRE). MRE live freely in the AMF cytoplasm, and have been found associated with all lineages of AMF worldwide. Virtually nothing is known about the MRE, such as their evolution, biological capabilities, and whether they are mutualists or parasites of their AMF hosts. In order to understand the nature of this symbiosis, and determine the role that the MRE play in arbuscular mycorrhizae, next generation sequencing (Roche 454 and Illumina) was performed on MRE isolated from 3 distinct AMF hosts, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Racocetra verrucosa*. Phylogenetic reconstruction and divergence dating using 22 conserved genes have revealed that MRE form a novel monophyletic subclade of the *Mycoplasmas* and have diverged from their *Mycoplasma* relatives at least 400 million years ago, which may indicate the establishment of the MRE-AMF association to be quite ancient. Analysis of annotated genes have revealed novel proteins that are likely to play a role in interacting directly with the fungal host. Preliminary data suggest that MRE are important in enabling the completion of the life cycle of their AMF hosts.

610. The Velvet gene is required for mutualism between *Epichloë festucae* and perennial ryegrass. Mostafa Rahnama[1,2], Richard Gardner[1], Damien Fleetwood[2]. 1) School of Biological Sciences, University of Auckland, Auckland, New Zealand; 2) Forage Improvement Group, AgResearch, Auckland, New Zealand.

The velvet gene (*veA* or *velA*) is a key factor in the regulation of fungal development, biosynthesis of secondary metabolites and hyphal growth. This study aimed to determine the role of *velA* regulation in *Epichloë festucae* and its mutualistic interaction with the agriculturally important forage perennial ryegrass (*Lolium perenne*). Infection of perennial ryegrass with an *E. festucae* mutant deleted in *velA* caused rapid seedling death in two thirds of infected plants while remaining plants displayed a normal interaction phenotype, although after several weeks these plants also become stunted and died in an unusual delayed plant-interaction phenotype. No hypersensitive response was observed by microscopy, suggesting the response is not driven by pathogen-like effector proteins. Microscopic analysis showed different accumulation of polysaccharides between mutant and wild type strains. The mutant strain could grow in higher concentrations of calcolfluor and also there was different colony hydrophobicity between wild type and mutant strains. These different cell wall properties suggest a possible microbe associated molecular pattern (MAMP)-triggered defense response may be occurring in D*velA* mutant associations. We are currently analysing the transcriptomes of wild type and mutant *E. festucae/Lolium perenne* symbiota to determine the *velA* regulon and elucidate the mechanism of host death.

611. An examination of phosphate solubilization and hormone production by two *Penicillium* species growing in the rhizoplane. Tim Repas[1,2], David Greenshields[2], Susan Kaminskyj[1]. 1) Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 2) Novozymes BioAg Ltd. 3935 Thatcher Ave, Saskatoon, SK S7R1A3, Canada.

Some soil microorganisms, including fungi, can enhance plant growth in the natural environment, however the mechanism(s) by which they promote plant growth (PGP) are only partly known, and at any rate these will likely vary between organisms. Possible mechanisms for PGP include enhanced nutrient uptake, hormone production, pathogen biocontrol, and increased water use efficiency. We are evaluating the potential of two rhizosphere fungi, *Penicillium bilaiae* (Pbil) and a novel isolate (Skj340) that is most closely related to *Penicillium atramentosum*. Both fungi can solubilize in liquid culture each of Fe-, Al-, and Ca-phosphates, which are commonly found in soil. Both strains were able to solubilize phosphate minerals equally well. However, Pbil produces abundant organic acids, whereas Skj340 does not produce organic acids, nor even change the pH of the spent medium. Pbil has been tagged with red fluorescent protein (RFP); Skj340 has been stained with lactofuschin; both were imaged with confocal fluorescence. Both fungi are found on the root surface, and neither could be isolated from surface sterilized plants, thus it appears that these strains are not endophytic. We are currently evaluating production of gibberellins and auxins from Pbil and Skj340 by assessing their ability to complement the phenotype of *Arabidopsis* mutants deficient in these hormones. We will also assess whether either strain enhanced root hairs counts, which is expected to be correlated with both nutrient uptake and hormone activity.

612. The role of *Epichloe festucae* RacA interacting proteins, PakA, PakB and RhoGDI, on cell polarity in culture and synchronized growth in *Lolium perenne*. Yvonne Becker, Carla Eaton, Isabelle Jourdain, Barry Scott. Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.

The fungal endophyte *Epichloe festucae* and its host *Lolium perenne* are an interesting model system to study signals and mechanisms involved in mutualistic symbiosis maintenance. Mutants defective in components of the ROS producing Nox complex show loss of synchronized growth of the fungus in the grass resulting in stunted, multi-tillered plants (Tanaka et al. 2006, Takemoto et al., 2006). The small GTPase RacA is crucial to activate the Nox complex in *E. festucae* and plays a crucial role in establishment and maintenance of polarized hyphal growth (Tanaka et al., 2008). The objectives of this study were to determine whether key regulators of RacA in mammalian systems, the guanine nucleotide dissociation inhibitor (RhoGDI) and p21-activated kinases (Paks), also regulate fungal RacA in order to control polarised growth in culture and Nox activity for maintenance of the symbiosis with perennial ryegrass. We showed by yeast two-hybrid analysis that PakA (Cla4 homolog), PakB (Ste20 homolog) and RhoGDI interact with RacA, whereas the RhoGDI interaction is compromised in a mutant of RacA (R73E) required for RhoGDI binding. Only partial complementation is achieved when RacA (R73E) is expressed in the RacA deletion strain, indicating RhoGDI is important for controlling RacA function. Deletion of *pakB* had a mild effect on polarized hyphal growth in culture and wild-type growth *in planta*. Deletion of *pakA* had a severe effect on polarized hyphal growth in culture, with a reduction of radial growth and hyper-branching, a phenotype similar to the *racA* mutant but surprisingly plants infected with the *pakA* mutant had a wild-type interaction phenotype. The *in planta* results may reflect the fact that *E. festucae* grows by intercalary rather than tip growth in the intercellular spaces of perennial ryegrass leaves.

613. A Host-Induced Gene Silencing Approach to Control Mycotoxin Contamination in Corn. J. E. Smith, Y. B. Ramegowda, B. H. Bluhm. University of Arkansas, Fayetteville, AR.

The fungal ear rot pathogens *Aspergillus flavus* and *Fusarium verticillioides* contaminate corn with aflatoxins and fumonisins, which pose severe health

risks and significantly limit grain marketability. Despite extensive breeding efforts, adequate resistance to mycotoxin accumulation has not been developed. Additionally, tools currently available to control mycotoxin contamination are limited in number and efficacy. Recently, host-induced gene silencing (HIGS) has emerged as a way to manipulate gene expression in fungal pathogens via expression of pathogen-specific hairpin RNA (hpRNA) in plants. The goal of this research was to generate transgenic corn inhibiting mycotoxin accumulation via HIGS. To this end, a high-throughput workflow was developed to create and validate HIGS expression vectors. First, candidate fungal genes regulating mycotoxin biosynthesis were identified through various approaches, including expression profiling and functional genomics. Second, a one-step cloning process was developed to simultaneously create hpRNA-encoding constructs comprised of sense and antisense orientations of target fungal genes, separated by an intron from the *Magnaporthe oryzae* cutinase gene, flanked by the TrpC promoter and terminator. Constructs were validated by phenotypic assessment after transformation into either *A. flavus* or *F. verticiliioides*. Finally, constructs encoding hpRNAs that significantly reduced mycotoxin accumulation were cloned into a plant expression vector and transformed into maize. Currently, silencing vectors have been created that target the a-amylase (*AMY1*) and hexokinase (*HXK1*) genes in *F. verticillioides* and the polyketide synthase (*aflC*) and hexokinase (*kxk*) genes in *A. flavus*, and transgenic corn plants have been created. Thus, this research will advance the current understanding of HIGS in maize and will ultimately provide new tools to control mycotoxin contamination of corn.

614. CDiT1, a novel type of proteinaceous toxin secreted by the necrotrophic pathogen of the roots of tomato *Pyrenochaeta lycopersici*. Pierre-Henri Clergeot[1], Herwig Schuler[2], Ejvind Mørtz[3], Maja Brus[1], Simina Vintila[1], Sophia Ekengren[1]. 1) Vaxtfysiologi, Stockholms Universitet, Stockholm, Sweden; 2) Karolinska Institutet, Stockholm, Sweden; 3) Alphalyse A/S, Odense, Denmark.

During the 24th Fungal Genetics Conference in Asilomar in 2009, we reported the isolation by Fast Protein Liquid Chromatography of a putative proteinaceous toxin of 18 kDa, secreted in liquid medium by the corky root rot pathogen of tomato, the filamentous ascomycete *Pyrenochaeta lycopersici*. This molecule, CDiT1, was thought to be the cause of cell death observed in tomato leaves after infiltration of culture filtrates into their apoplast. Further characterization of CDiT1 revealed that it is secreted as a dimer and encoded by a single gene, whose expression peaks during tomato root infection. Infiltration into leaves of various hosts of *P. lycopersici* of recombinant CDiT1 purified by affinity confirmed its phytotoxic, but differential activity. Especially, currant tomato (*Solanum pimpinellifolium*) proved to be tolerant to a higher concentration of recombinant CDiT1 than cultivated tomato (*S. lycopersicum*). This correlates with the observation that roots of currant tomato are less prone to intracellular infection by a transformant of the fungus expressing a reporter gene than those of cultivated tomato. Affinity-purified recombinant CDiT1 was also used in cut root assays to confirm lethal activity of the toxin on tomato root cells. Finally, searches made by sequence similarity in the genomes of other pathogenic Pleosporales showed that CDiT1 has a putative orthologue in the cereals pathogens *Stagonospora nodorum*, *Pyrenophora teres f.sp. teres* and *Pyrenophora tritici-repentis*, species known for secreting proteinaceous toxins contributing to virulence as well. In conclusion, our data validate the experimental approach of characterizing molecules secreted by *Pyrenochaeta lycopersici* and inducing disease-related symptoms after infiltration into host leaves, this in order to highlight potential genetic resistance against corky root rot in related species or varieties (see also Clergeot et al. 2012, Phytopathology, 102:878-891).

615. Nonhost-specific phytotoxicity of the polyketide-derived toxin solanapyrone A produced by *Ascochyta rabiei* and *Alternaria solani*. W. Kim[1], L. Tymon[1], D. Johnson[1], W. Chen[2]. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Grain Legume Genetic and Physiology Research Unit, Pullman, WA.

Solanapyrone A is a polyketide-derived metabolite produced by *Ascochyta rabiei* and *Alternaria solani*, which are the most destructive necrotrophic pathogens of chickpea and potato/tomato, respectively. They belong to the Order Pleosporales within the Class Dothideomycetes, but are phylogenetically distantly-related. All isolates of the two fungi tested so far are capable of producing solanapyrone A in synthetic media, which may imply that it is indispensable for their life cycle. However, very little is known about the genetics of solanapyrone A production and its role in pathogenesis and their life cycle. Recently, solanapyrone biosynthesis gene cluster was identified in *Al. solani*. Six genes (*Sol1 - Sol6*) form the gene cluster, spaning about 20 kb of the genome. Among them, *Sol5* gene encodes a Diel-Alderase which catalyzes the final step of solanapyrone biosynthesis pathway. Knockout of the *Sol5* gene in both *A. rabiei* and *Al.solani* resulted in the production of three compounds (presumably solanapyrone precursors), instead of solanapyrone A. Colony of *sol5* mutants showed expansive growth in agar medium until covering the entire plates, in contrast to restricted growth of colony of their corresponding wild-type progenitors. The restricted growth of the wild type strains is likely due to solanapyrone toxicity. Phytotoxicity of solanapyrone A was examined with various plant species including their natural host plants. Solanapyrone A produced similar size of necrotic lesions on all plant species tested. On the other hand, one of the putative solanapyrone precursors with the same molecular weight of solanapyrone A caused much smaller lesion only around the wounds of application sites. These results indicate that solanapyrone A is a nonhost-specific phytotoxin because it caused similar degree of lesions on host and nonhost species.

616. Crosstalk of the unfolded protein response and regulatory pathways controlling pathogenic development in *Ustilago maydis*. Kai Heimel[1], Johannes Freitag[2], Martin Hampel[1], Julia Ast[2], Michael Bölker[2], Jörg Kämper[3]. 1) Department of Molecular Microbiology and Genetics, Georg-August-University, Göttingen, Germany; 2) Genetics Department, Philipps-University, Marburg, Germany; 3) Genetics Department, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

Development of eukaryotic pathogens is accompanied by dramatic changes in morphology, lifestyle, nutrient acquisition and growth behavior. Consequently, pathogens require robust control systems to adapt to changing host environments and to maintain cellular physiology and homeostasis. The unfolded protein response (UPR) is a conserved eukaryotic signaling pathway counteracting endoplasmic reticulum (ER) stress during situations of increased demands on the secretory pathway. We identified and characterized the homologs of the central UPR regulators, Hac1 and Ire1 in the biotrophic fungus *U. maydis*. The UPR is tightly interlinked with the *b* mating-type-dependent signaling pathway that regulates pathogenic development. Exact timing of UPR is required for virulence and premature activation interferes with the *b*-dependent switch from budding to filamentous growth. A smut-specific C-terminal extension of the *U. maydis* Hac1 homolog, Cib1, mediates direct interaction with Clp1, an essential component of the *b*-mediated signaling cascade. This interaction leads to stabilization of Clp1, increased ER stress resistance and thus prevents deleterious hyperactivation of the UPR during biotrophic growth of *U. maydis*. Since Clp1 expression is decisive for cell cycle release and fungal proliferation *in planta* we suggest that UPR activation serves as a checkpoint to time developmental progression and secretion of effector molecules, which promote the establishment of the biotrophic interaction.

617. Transcriptional profiling of the APSES genes in Trichophyton rubrum during growth on human nail. Elza A. S. Lang[1], Nalu T. A. Peres[1], Maíra P. Martins[1], Tiago R. Jacob[1], Pablo R. Sanches[1], Larissa G. Silva[1], Antonio Rossi[2], Nilce M. Martinez-Rossi[1]. 1) Department of Genetics, Ribeirao Preto School of Medicine, University of Sao Paulo, Brazil; 2) Department of Biochemistry and Immunology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil.

The dermatophyte Trichophyton rubrum is worldwide spread and is the most prevalent causative agent of clinical cases of skin and nail mycoses in humans. Adhesion, invasion and colonization of keratinized host tissues are crucial for the success of the infection process and depend on the modulation of genetic responses during host-pathogen interactions. The APSES transcription factors are exclusive of the fungi kingdom and have been reported to play important roles in cell growth, differentiation, pathogenicity and virulence in several fungi species. In this work, in silico analyses of T. rubrum genome revealed the presence of genes encoding distinct proteins containing the APSES domain, suggesting that these proteins may play different roles in the cell. A high number of genes potentially regulated by the APSES regulators were identified by in silico analyses of 1000nt upstream of the annotated ORFs of T. rubrum. Transcriptional profiles of the APSES genes were analyzed during growth of T. rubrum on human nail or keratin, as the sole source of nutrients. In vitro infection of human nail was also evaluated by light microscopy. The results revealed that the transcription levels of the APSES genes are modulated during the human nail infection process and keratin degradation. Taken together, our findings suggest that the APSES genes of T. rubrum may be implicated in host-pathogen interactions. Financial support: FAPESP, CAPES, CNPq, FAEPA.

618. Carbohydrate binding proteins of two *Leptosphaeria* pathogens of *Brassica napus*. Rohan G T Lowe[1], Bethany Clark[1], Angela Van de Wouw[1], Andrew Cassin[1], Jonathan Grandaubert[2], Thierry Rouxel[2], Barbara Howlett[2]. 1) School of Botany, University of Melbourne, Melbourne, Victoria, Australia; 2) INRA-Bioger, Campus AgroParisTech, Thiverval-Grignon, France.

Effectors include small secreted proteins (SSPs) produced by pathogens to modify or subvert defence responses of the host organism. *Leptosphaeria maculans* “brassicae”, the foremost pathogen of *Brassica napus* (canola), has 651 genes predicted to encode SSPs. The related species *L. biglobosa* "canadensis" more aggressively infects *Brassica* cotyledons, but causes fewer stem cankers. This difference in symptomology may be due to a different response from the host innate immune system. Compared to the *L. maculans “brassicae”* v23.1.3 reference genome, *L. biglobosa* has a relatively compact genome (30 Mbp) lacking the characteristic AT-rich, gene-poor repeats of *L. maculans*. We are using comparative transcriptomics to identify genes involved in early stages of infection. RNAseq analysis revealed that >300 *L. maculans* “brassicae” genes are highly upregulated (>100-fold) 7 days after infection compared to in vitro growth. These genes are enriched for SSPs, which comprise 25 of the top 100, but only 5% of the total gene complement. A major class of SSPs are carbohydrate active enzymes (CAZY), some of which play a role in evasion of chitin-triggered immunity in plants. *L. maculans* “brassicae”and *L. biglobosa* "canadensis" have different complements of the chitin-associated CAZY domains, CBM18 and CBM50 (aka LysM). Both of these domains bind chitin or peptidoglycan and may be found with chitinase domains in the same protein. The genomes of both *Leptosphaeria* species encode a similar number of CBM18 domains (27 and 29, respectively). *L. maculans* “brassicae” has predominantly multi-domain CBM18 proteins that are not highly upregulated 7 days after infection, whilst *L. biglobosa* "canadensis" has more abundant single domain proteins, two of which are highly upregulated (>100-fold) at 7 days post inoculation. Homologs of the well-characterised CBM50/LysM protein, ECP6, from *Cladosporium fulvum* are present in both species, and a triplication of the N terminal LysM domain has occurred in *L. maculans* “brassicae”. The composition and regulation of CBM18 and CBM50-containing genes in Leptosphaeria may be involved in determining the degree of chitin-triggered immunity in the host canola, and concomitantly, the success of the pathogen. Silencing of key CAZY genes in *L. maculans* “brassicae” is underway, with a focus on members of the CBM50/LysM family.

619. Domains for plant uptake of Ustilago maydis secreted effectors. Anupama Ghosh, Armin Djamei, Shigeyuki Tanaka, Regine Kahmann. Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-Von-Frisch-Strasse 10, D-35043 Marburg, Germany.

The genome of the corn smut fungus Ustilago maydis codes for a large repertoire of secreted effectors. Some of them play crucial roles for virulence and establishment of the biotrophic phase. The chorismate mutase Cmu1 is one such secreted translocated effector of U. maydis. cmu1 deletion strains are attenuated in virulence that is attributed to higher salicylate levels in plants infected with the mutant strain, most likely through alterations in the channeling of chorismate from the plastids to the cytosol. Here we identify the motif in Cmu1 that is necessary for the translocation of the protein across the plant plasma membrane and present a mutational analysis of this region. To test for uptake we assayed the ability of mutant proteins to complement a cmu1 mutant strain as well as the retained ability to complement the growth defect of a Daro7 strain of S. cerevisiae in minimal medium. By deletion analysis a region of 20 amino acids adjacent to the signal peptide was shown to be essential for the translocation. Microscopic analysis of maize tissue infected with U. maydis strains expressing Cmu1-mcherry fusion proteins with or without the probable uptake motif revealed that the 20 amino acid motif allows binding of the protein to an as yet unknown plant plasma membrane component. We hypothesize that the translocation of Cmu1 across the plant plasma membrane is a two step process; initiated by binding followed by translocation across the membrane. In addition, we present results where the 20 amino acid motif is substituted by motifs from other effectors.

620. Lipid metabolism influences virulence in *Ustilago maydis*. Scott Lambie, Matthias Kretschmer, Jim Kronstad. Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.

Plant tissues and surfaces are a source of lipids which act as a potential carbon source and signals for the pathogenic development of the biotrophic smut fungus *Ustilago maydis*. This pathogen is an excellent model for the molecular genetic analysis of lipid use during disease and responds to lipids with a morphologic transition from budding to filamentous growth. In addition, the fungus possesses both peroxisomal and mitochondrial b-oxidation pathways, and numerous putative phospholipases (PLs), to exploit lipid carbon sources for nutritional and signalling purposes. We have shown that both mitochondrial and peroxisomal b-oxidation is important for the utilization of fatty acids and the pathogenic development of *U. maydis* and may therefore represent a potential target to combat crop disease caused by fungal pathogens. In *U. maydis*, deletion of components of these pathways influenced mating, lead to a decrease in virulence, caused a defect in fatty acid metabolism, a loss of acetate metabolism and the accumulation of toxic intermediates. To further explore the role of the b-oxidation pathway during morphogenesis and pathogenic development, we have investigated the effects of several non-steroidal anti-inflammatory drugs (NSAIDs), which are known to interfere with b-oxidation functions at various stages. Diclofenac inhibited the usage of fatty acids of different chain length and saturation state as sole carbon sources and had an influence on the filamentation efficiency of those fatty acids. Further it showed fungicidal activity by inducing apoptosis, and influenced mating and pathogenic development. In an attempt to further elucidate the role of lipid utilization during infection we are investigating the role of PLs as a potential mechanism by which host-derived lipid signals or fatty acids for subsequent b-oxidation are generated. We present an analysis of 17 candidate PL genes identified by genome mining, as well as a preliminary functional characterization of these genes during mating and infection. Overall, our work demonstrates the utilization of host-lipids by *U. maydis* as an important nutritional and signalling source that is required for pathogenic development. Furthermore, we have begun to elucidate the underlying mechanisms involved which represent a potential target to combat crop diseases caused by fungi.

621. Functional characterization of the putative cell surface receptor for hydrophobicity, Msb2, in *Ustilago maydis*. Marino Moretti, Daniel Lanver, Irina L. Schmidt, Regine Kahmann. MPI for Terrestrial Microbiology, Marburg, Germany.

Msb2 is a transmembrane mucin protein involved in plant surface sensing in *U. maydis*. *Msb2* deletion mutants are defective in sensing the hydrophobic

leaf surface which is a prerequisite for the differentiation of infection structures. Consequently, *msb2* mutants are attenuated in virulence (Lanver *et al.*, 2010). The molecular mechanism leading to an activation of Msb2 and the downstream MAP kinase cascade is so far unknown. In yeast Msb2p is processed by the aspartyl protease Yps1p leading to an active cell-associated form and a secreted glycosylated part which has an inhibitory function in the full length protein. In *U. maydis* Msb2 is also processed, but so far there is no evidence that this leads to an activation of surface sensing. Using a yeast mutant lacking five aspartyl proteases we could demonstrate that yeast Yps1p is able to cleave *U. maydis* Msb2. In addition, by using this heterologous system, two *U. maydis* aspartyl proteases were identified that were weakly able to cleave Msb2. The respective genes were deleted in the solopathogenic strain SG200 and its *Dmsb2* derivative expressing Msb2-HA-GFP. Possible phenotypic alterations in virulence as well as in Msb2 processing will be monitored. In addition, a synthetic codon-adapted *YSP1* gene has been introduced in the above-mentioned *U. maydis* strains to analyze the effects of an increment in Msb2 cleavage on biological activity of the protein. Finally, the extracellular domain of Msb2 was subjected to a mutational analysis to identify regions with a presumed positive regulatory function. Lanver, D., Mendoza-Mendoza, A., Brachmann, A. and Kahmann, R. (2010). Sho1 and Msb2-Related Proteins Regulate Appressorium Development in the Smut Fungus *Ustilago maydis*. The Plant Cell *22*, 2085-2101.

622. The *U. maydis* effector Pit2 inhibits maize cysteine proteases to suppress host defense. Andre Mueller[1], Sebastian Ziemann[1], Steffi Treitschke[2], Daniela Abmann[1], Gunther Doehlemann[1]. 1) MPI for Terrestrial Microbiology, Karl-von-Frisch-Strabe 10, 35043 Marburg, Germany; 2) Fraunhofer ITEM-R, Biopark I, Josef-Engert-Strabe 9, 93053 Regensburg, Germany.

The basidiomycete *Ustilago maydis* is the causal agent of smut disease in maize. Infected plants show tumor formation in all infected aerial parts as prominent symptoms. As a biotroph pathogen, *U. maydis* depends on living plant tissue and hence efficient suppression of plant immunity is required. Therefore, infectious hyphae secrete effector proteins that interfere with specific components of the plant immune system. One such secreted effector-protein is Pit2 (**P**rotein **i**mportant for **t**umor-formation **2**), which, in a previous study, was found to be essential for tumor formation in infected plants [1]. Instead of tumors, necroses can be observed at infection sites indicating that plant defense and cell death reactions are triggered in D*pit2* infections [1]. Using a combination of yeast-two-hybrid- and protease activity assays, we could show that Pit2 acts as an inhibitor of apoplastic plant cysteine proteases whose activity is directly linked with salicylic acid (SA)-associated plant defenses. Sequence comparisons with Pit2 orthologs from related smut fungi identified a conserved 14 amino acid motif. Mutation of this motif leads to a loss-of-function of Pit2 and consequently to avirulence of *U. maydis*, suggesting that the protease inhibition by Pit2 is essential for plant infection. Moreover, synthetic peptides of the conserved motif show full activity as protease inhibitor. Interestingly, expression of only this motif in *U. maydis* partially restores virulence of the D*pit2*-mutant, substantiating the important role of this novel protease inhibitor in suppression of host immunity. [1] Doehlemann *et al*. 2011. Mol Micobiol 81: 751-766.

623. The Ustilago maydis MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-peptide enrichment. Vikram Naik[1], Gerold J.M. Beckers[2], Wolfgang Hoehenwarter[3], Regine Kahmann[1]. 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Plant Biochemistry and Molecular Biology Group, RWTH-Aachen University, Aachen; Germany; 3) Department for Molecular Systems Biology, Faculty of Life Sciences, University of Vienna, Vienna, Austria.

In the plant pathogenic fungus Ustilago maydis three MAP kinase modules have been identified mostly via their homology to genes in Saccharomyces cerevisiae. The module consisting of the MAP kinase kpp2, the MAP kinase kinase fuz7 and the MAP kinase kinase kinase kpp4 controls pheromone signalling and plays an essential role in mating and pathogenicity. Kpp2 is involved in filamentation and appressorium development while the MAP kinase, Kpp6, which also acts downstream of Fuz7, is required for appressorial penetration of plant epidermal cells. Our goal is to identify crucial virulence factors which act directly downstream of the MAP kinases Kpp2 and Kpp6. For this we generated a strain in which MAP kinase signaling can be induced by expressing a constitutively active version of the MAPKK Fuz7 (Fuz7DD) under an inducible promoter in the presence or absence of kpp2 and kpp6. We then used a two-step chromatographic procedure combining phosphoprotein enrichment using Al(OH)3-based metal oxide affinity chromatography (MOAC), followed by tryptic digest of enriched phosphoproteins, and TiO2-based MOAC for phosphopeptide enrichment. This enabled detection of low abundant phosphorylated peptides using LC-MS/MS and allowed direct identification and site-specific quantification of phosphorylated peptides that differentially accumulated after MAP kinase activation in wild type and mutant cells. LC-MS/MS analysis of the phosphopeptide fraction obtained after the two-step MOAC yielded 111 putative substrates of Kpp2 and Kpp6 MAP kinases in three replicate experiments. Of these 20 differentially phosphorylated proteins were chosen for subsequent functional analyses. We are presently generating deletion mutants of these genes in compatible U. maydis strains that carry different a and b alleles and in a solopathogenic strain. In addition, we are analysing the expression pattern of the chosen genes during the different developmental stages of U. maydis. Results on the role of these U.maydis genes on signaling and pathogenicity will be presented.

624. See1 : A novel organ specific effector in the *Ustilago maydis* - maize interaction. Amey Redkar[1], Christoph Hemetsberger[1], Ziba Ajami-Rashidi[1], Virginia Walbot[2], Gunther Doehlemann[1]. 1) Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl von Frisch Strasse 10, Marburg, 35043 Germany; 2) Department of Biology, Stanford University, Stanford, California. 94305-5020 USA.

Ustilago maydis is a biotrophic smut fungus which infects all aerial organs of its host plant maize. The disease progression and development of infection is by reprogramming of the plant tissue which ultimately results in formation of tumors. This tumor induction is likely being triggered by small secreted proteins by the fungus, so called effectors. Given the fundamental differences between the different maize organs that are colonized by *U. maydis*, we hypothesized that the fungus deploys organ specific effectors to manipulate physiology and development of specific host tissues (1). To further investigate the role of individual organ specific effectors in modulating biotrophy, we in the present study identified a novel secreted protein, termed See1 (**S**eedling **e**fficient **e**ffector 1) that is strongly induced in seedling leaves but only weakly expressed in tassels and ears. *U. maydis* deletion mutants for *see1* show a strong reduction of tumor formation in maize seedlings but not in floral tissues. Laser scanning confocal microscopy shows that the mutant hyphae successfully enter the leaf tissue but might be blocked during pre proliferation stages in the mesophyll tissue of the leaf. Moreover, by labeling replicating DNA by 5-ethynyl-2'-deoxyuridine (EdU) we observed that maize seedling colonized by *Dsee1* do not show mitotic activity during infection, while cell division in leaves is specifically induced in wildtype infected host cells. In contrast, the *Dsee1* mutant induces normal tumor formation in tassels and also shows the stable cell division rate in colonized anthers. Overexpression of *see1* causes a hypervirulent phenotype only in the vegetative parts of the tassel, which are not transformed to tumors in wild type infections. To localize See1 during the disease progression we are applying confocal microscopy with live cell imaging using mCherry-tagged See1 protein. Most importantly, we are aiming for the identification of *see1* interaction partners to link the observed phenotypes with its molecular function to understand its organ-specific function for *U. maydis* virulence. (1) Skibbe D*, Doehlemann G*, Fernandes J, Walbot V. (2010) Maize tumors caused by *Ustilago maydis* require organ-specific genes in host and pathogen. *Science* 328:89-92.

625. Investigation of unconventionally secreted proteins in *Ustilago maydis*. Stefanie Reissmann[1], Sina Krombach[1], Florian Bochen[1], Till Ringel[1], Saskia Kreibich[1], Thomas Brefort[1], Kerstin Schipper[1,2], Matthias Mann[3], Regine Kahmann[1]. 1) Organismic Interactions, Max Planck Institute for Terrestrial

Microbiology, Marburg, Hessen, Germany; 2) Heinrich Heine University Düsseldorf, Institute for Microbiology, Universitätsstrabe 1, 40225 Düsseldorf; 3) Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany.

Secreted fungal proteins play a crucial role during the biotrophic interaction between the smut fungus *Ustilago maydis* and its host plant *Zea mays*. In the last decade it has been well established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/ Golgi independent manner. We want to identify such unconventionally secreted proteins in *U. maydis* and investigate their potential function as pathogenicity factors. Our approach is based on affinity purification of tagged candidate proteins, detected in the apoplastic fluid of infected maize leaves. Four of twelve candidate proteins tested so far could be detected in culture supernatants. One candidate protein, Um11938, displays 55 % amino acid similarity to the human sterol carrier protein 2 (SCP2) and we were able to demonstrate that it co-localizes intracellularly with peroxisomes. Mammalian SCP2 is detected in peroxisomes but also found in the cytoplasm. It interacts with a variety of phospholipids as well as cholesterol and has been implicated in non vesicular cholesterol transport and in regulating lipid rafts (Schroeder *et al.*, 2007). *um11938* deletion strains of *U. maydis* are severely compromised in virulence and peroxisomal localization of Um11938 is required to fulfill its function as a pathogenicity factor. *um11938* deletion strains display no growth defect on minimal media supplemented with different fatty acids as sole carbon source, suggesting that the Um11938 protein is not involved in *bona fide* b-oxidation. The results of ongoing experiments aimed to differentiate whether the pathogenicity relevant function of Um11938 is performed extracellularly or within the fungal peroxisomes will also be presented.

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626. Identification of a key regulator for the developmental switch leading to sporogenesis in *Ustilago maydis*. Marie Tollot, Regine Kahmann. Organismic Interactions, MPI Marburg, Marburg, Hessen, Germany.

Ustilago maydis is a biotrophic pathogen of maize. Its life cycle begins with the mating of two compatible haploid sporidia to form a dikaryotic infectious filament. After penetrating the plant cuticle, the dikaryon spreads inside the plant tissues and induces the formation of tumors. At a defined time of development, the sporogenesis program begins in tumor tissue: the hyphae start to fragment into individual cells that eventually differentiate into mature teliospores. The isolation of several mutants affected in spore formation has shown that a tight regulation of the cAMP signaling pathway and the activity of two transcriptional regulators Hda1 and Rum1, potentially functioning in the same chromatin modifying complex, are required. We have identified a new regulator for the sporogenesis program of *U. maydis*. It belongs to a family of transcriptional regulators that are characterized by the presence of a WOPR DNA binding domain. The best studied member of this family is Wor1 from *Candida albicans* which plays a key role in the switch from the non-pathogenic to the pathogenic form of the fungus. The *U. maydis wor1* homologue *um05853*, was deleted in the compatible haploid strains FB1 and FB2. The deletion mutants were able to mate and to infect maize as efficiently as the wild-type. However, although the tumor rate was similar, no spores could be detected in plants infected by the deletion mutants. Confocal microscopy of the mutant dikaryon revealed that hyphal fragmentation and consequently spore maturation were not occurring. The hyphae were still spreading at a time when the wild type dikaryon had already formed mature spores. To further investigate the role of *um05853*, we expressed the gene in a haploid strain where the filamentous program can be induced in axenic culture via expression of a functional b-heterodimer. The cells failed to switch to filaments, started to enlarge and showed septation, with each section containing one nucleus. This result suggests that Um05853 is able to counteract the b function and trigger fragmentation. Preliminary results show that Um05853 might downregulate several b-dependent genes including the gene encoding the master regulator Rbf1. We are currently identifying targets of Um05853 using a microarray approach and expect that these results will highlight how Um05853 controls spore formation.

627. Genetic characterization of virulence in the *Pyrenophora teres* f. *teres* - barley pathosystem. Timothy L. Friesen[1,2], Rachel A. Shjerve[2], Justin D. Faris[1], Robert S. Brueggeman[2]. 1) Cereal Crops Research Unit, USDA-ARS, Fargo, ND; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND.

Pyrenophora teres f. *teres* is a necrotrophic fungal pathogen that causes net form net blotch (NFNB) on barley throughout the world. Several resistance sources have been identified but few are effective against all *P. teres* f. *teres* pathotypes, indicating that the pathogen has an arsenal of effectors that are involved in disease induction. Genetic analysis identified two barley genotypes, Rika and Kombar, that each harbor unique dominant susceptibility genes located at the centromeric region of barley chromosome 6H. *P. teres* f. *teres* isolate 15A is virulent on Kombar but avirulent on Rika whereas *P. teres* f. *teres* isolate 6A is virulent on Rika but avirulent on Kombar. Based on the necrotrophic effector model, 15A and 6A each secrete unique effectors that are directly or indirectly interacting with genes on chromosome 6H in Kombar and Rika, respectively. A linkage map was generated using a mapping population developed from a sexual cross between 15A and 6A, and 118 progeny were phenotyped on barley genotypes Rika and Kombar. Two major virulence QTL derived from 15A contributed to virulence on Kombar, and two additional unique virulence QTL derived from 6A contributed to virulence on Rika. All susceptibility loci in the host mapped to the same region on barley chromosome 6H. Therefore, it is likely that at least four necrotrophic effectors present throughout the *P. teres* f. *teres* genome are interacting with host susceptibility genes located in one region of barley chromosome 6H. These results strongly indicate that the NFNB-barley compatibility is at least partially due to necrotrophic effector-host susceptibility gene interactions that result in disease induction. Currently, we are using a genotype by sequencing (GBS) approach to generate a saturated map to identify candidate genes in the QTL regions in order to clone and characterize the effector genes involved in the NFNB interaction.

628. Illumina-based genetic linkage map for wheat leaf rust. David L. Joly[1,2], Barbara Mulock[3], Christina A. Cuomo[4], Barry J. Saville[2], Brent D. McCallum[3], Guus Bakkeren[2]. 1) Pacific Agri-Food Research Centre, Agriculutre and Agri-Food Canada, Summerland, British Columbia, Canada; 2) Forensic Science Program and Environmental & Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada; 3) Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada; 4) Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA 02142.

Few genetic maps have been made for rust fungi; yet they are useful in identifying candidate loci for phenotypic traits or in unravelling chromosomal arrangements. This lack of maps is, in part, due to the obligate biotrophic nature of rusts and the difficulties in manipulating their life cycle in a way that enables controlled crosses. Recently, the genome sequence of a wheat leaf rust (Puccinia triticina) isolate was determined and this prompted the sequencing of additional isolates using next-generation sequencing technologies. This has dramatically increased the amount of sequence information available at a substantially decreased per base cost. Fifty-seven F2 progeny of a wheat leaf rust sexual cross between race 9 (SBDG) and race 161 (FBDJ) were sequenced using Illumina. In order to generate a high-resolution genetic linkage map, genome-wide single-nucleotide polymorphisms (SNPs) were identified. Employing the genome sequence information from the two parents and the F1 isolate, more than 25,000 SNPs were selected and used to generate a genetic linkage map. Although they were obtained from different isolates, the genetic map and the reference genome were integrated, allowing the creation of pseudomolecules. Those represent a strong improvement over the currently fragmented status of the reference genome. Moreover, at least 9 seedling and 2 adult-plant avirulence genes were shown to segregate in this F2 population and candidate genes identified using the genetic map are currently being investigated.

629. The deletion of the *Histoplasma capsulatum RYP1* homolog in *Coccidioides posadasii* is avirulent. M Alejandra Mandel[1,3,4], Hien Trien[2,3,4], Amritha Wickramage[1], Lisa Shubitz[2,3,4], Marc Orbach[1,3,4]. 1) School of Plant Sciences, University of Arizona, Tucson, AZ; 2) Department of Veterinary Sciences and Microbiology, University of Arizona, Tucson, AZ; 3) The Bio5 Institute, University of Arizona, Tucson, AZ; 4) Valley Fever Center for Excellence, Tucson, AZ.

Coccidioides spp. are mammalian fungal pathogens endemic to the desert southwestern US, parts of México and Central and South America that cause the respiratory disease coccidioidomycosis, or valley fever. These dimorphic fungi grow as filamentous saprotrophs in soil, but when a spore is inhaled by the host and localizes to the lung, it switches from polar to isotropic growth resulting in the development of a spherule. In *Histoplasma capsulatum*, Ryp1 is a master switch required for the transition from the filamentous to the infectious yeast phase, and thus is essential for virulence. We have performed a whole-gene deletion of the *RYP1* homolog in *Coccididoides posadasii* strain Silveira to determine whether it plays a similar role in virulence in this pathogen. Phenotypic effects were observed in both the filamentous and the parasitic phases of *C. posadasii*. During filamentous growth, there is a reduction in colony size, and defects in sporulation. The mutant is avirulent in our susceptible mouse model. Our results indicate that Ryp1 is a master switch in different fungal models. Although avirulent, the ryp1 mutant is not able to induce a protective response when used to vaccinate mice prior to wild type infection.

630. Cpkk2, a MEK from *Cryphonectria parasitica* is necessary for maintenance of CHV1 virus infection. M. Moretti, M. Rossi, M. Ciuffo, S. Abba', M. Turina. IVV, CNR, Torino, Italy.

We have recently obtained and characterized the knock out strains of the three MEKs present in the *Cryphonectria parasitica* genome, Cpkk1, Cpkk2 and Cpkk3, homologues of yeast Mkk1p/Mkk2p, Ste7p and Pbs2p, respectively. We tried to infect each of the knock-out strain with *Cryphonectria hypovirus 1* (CHV1), a mycovirus causing hypovirulence: Dcpkk1 and Dcpkk3 were easily infected by CHV1 through anastomosis, but we failed to infect Dcpkk2. We then showed that hyphal fusion was prevented in such knock-out strain: for this reason we attempted at infecting the Dcpkk2 strain with two alternative protocols that overcome the hyphal phusion impairment: stable transformation of protoplasts with a cDNA infectious clone and transfection of protoplasts with viral RNA transcripts obtained in vitro from a cDNA infectious clone. We originated infected strains with both protocols using wild-type *C. parasitica* protoplasts, whereas no stable infected strain was obtained starting from Dcpkk2 protoplasts, which, on the contrary, could be transformed with the empty vector carrying only the resistance gene for selection. Given the uniqueness of such result, we are now trying to show what is the specific molecular impairment that prevents CHV1 maintenance in Dcpkk2 strain. A proteomic approach was undertaken using 2-DE MALDI-TOF MS/MS and shotgun coupled to LC-MS/MS to compare the WT and Dcpkk2 strains. A number of metabolic pathways are heavily impacted in the mutant. Of interest, proteins involved in folding, transport and trafficking, are up-regulated suggesting an altered protein turnover. Defence machinery is also up-regulated, indicating that the fungus perceives a stress situation. Moreover, a strong down-regulation of proteins involved in energy production and conversion was detected, indicating a possible reduction of the energetic metabolism. Among them are some GAPDH isoforms. Given the recent discovery of the role of GAPDH in viral replication complexes of RNA viruses, we obtained anti-GAPDH antibodies in order to study its possible role in CHV1 viral replication.

631. Deep RNAseq of wheat leaf infection by M. graminicola identifies phase-specific in planta expressed genes and varying transcriptional contributions of fungal chromosomes. Jason J Rudd[1], Juliet Motteram[1], Mark Derbyshire[1], Keywan Hassani-Pak[2], Bob Dietrich[3], Arvind K Bharti[4], Andrew D Farmer[4], Ambrose Andongabo[2], Mansoor Saqi[2], Mikaël S Courbot[5]. 1) Rothamsted Research, Department of Plant Biology and Crop Science, Harpenden, Hertfordshire, AL5 2JQ, UK; 2) Rothamsted Research, Department of Computational and Systems Biology, Harpenden, Hertfordshire, AL5 2JQ, UK; 3) Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Durham, NC 27709, USA; 4) National Center for Genome Resources (NCGR), Santa Fe, NM 87505, USA; 5) Syngenta Crop Protection Münchwilen, Schaffhauserstrasse, 4332 Stein, CH.

Mycosphaerella graminicola is the causal agent of Septoria tritici blotch disease of wheat. Infection of leaves by M. graminicola involves a characteristic long period of symptomless intercellular growth of at least 8-10 days prior to the formation of necrotic leaf lesions. The genome sequence of the model isolate of M. graminicola, IPO323, was recently published by the research community in conjunction with the JGI and has been shown to contain 21 chromosomes. We have performed a deep RNAseq analysis to investigate fungal gene expression in vitro (in Czapek-Dox (CDB) and Potato Dextrose broth) and throughout phases of plant infection: day 1 (d1) germination on the leaf surface, day 4 (d4) slow growth in the absence of symptoms within the leaf, day 9 (d9) symptoms of disease become visible, day 14 fungal growth rate increases and finally day 21 when the fungus is sporulating asexually in fully necrotic plant tissue. Sequencing was performed on the Illumina Hiseq platform. The RNA-seq data was analysed using the Tuxedo tools (Trapnell et al., 2012). Tophat2 was used to map the reads against the M. graminicola genome. Transcript abundance (in FPKM) was determined using Cufflinks. Significant changes in transcript expression across all 21 pairwise comparisons were determined using cuffdiff (FDR<0.05, p-value <0.01). Remarkably even by d1 of plant infection > 600 differentially expressed genes were detected relative to growth in CDB culture. Many of the most strongly expressed genes from d1 to d9 of infection encode predicted secreted proteins, mostly of unknown function. No genes whatsoever displayed differential expression between d4 and d9 of infection and only 1 between d1 and d4. This implies that the fungus responds to the plant early and maintains a consistent level of gene expression throughout early symptomless infection. Amongst the genes up-regulated specifically in planta, were those that appeared to be present in gene clusters surrounding polyketide synthases, suggesting the up-regulation of specific secondary metabolites during infection. Finally overall read mapping to chromosomes highlighted the fact that the eight smallest chromosomes (Chr14-21) were significantly less transcriptionally active than the 13 larger “core”

chromosomes, which may be consistent with their being dispensable for asexual plant infection.

632. A genomic analysis of the infection strategies employed by *Phoma medicaginis* a necrotrophic fungal pathogen of alfalfa and the model legume *Medicago truncatula*. Angela H. Williams[1,4], James K. Hane[2], Robert D. Trengove[3], Karam B. Singh[2], Richard P. Oliver[4], Judith Lichtenzveig[4]. 1) Murdoch University, Perth, Australia; 2) CSIRO Plant Industry, Perth, Australia; 3) Separation Science and Metabolomics Laboratory, Murdoch University, Perth, Australia; 4) Department of Environment and Agriculture and the Australian Centre for Necrotrophic Fungal Pathogens, Curtin University, Perth, Australia.

Phoma medicaginis is a necrotrophic plant pathogen that causes black spot of alfalfa (*Medicago sativa*) and the closely related model legume *Medicago truncatula*. It is a member of the Didymellaceae family, a distinct clade within the order Pleosporales which includes some of the most important pathogens of legume crops. We present here the first genome assembly of *P. medicaginis* and the results of investigations into the host-pathogen interaction, focusing on identification of necrotrophic effectors (NEs) using a combination of proteogenomic and transcriptomic analyses. A draft genome assembly was constructed using Illumina paired-end reads, *de novo* assembled into 952 nuclear scaffolds totaling 31.4 Mbp, with ~27 x coverage and encoding ~10,500 predicted proteins (>50 amino acids) . Of these, ~1,000 are predicted to be secreted. Peptide sequencing via mass spectrometry was conducted in order to validate the gene set and characterise the protein content of intracellular and necrosis-inducing secreted fractions. This enabled the confirmation of 554 predicted genes and identified 162 proteins in the necrosis-inducing secreted fraction. To further validate the predicted gene set and examine differences in gene expression, the transcriptome was sequenced via RNA-seq at four important lifestyle phases. These included: 1) 1-5 days post infection of *M. truncatula*; 2) vegetative growth *in vitro*; 3) sporulation *in vitro* and 4) during growth in media where the culture filtrate produces necrosis and chlorosis when infiltrated into the plant. Close to 10,000 genes were expressed under one or more of these conditions with ~ 3,000 showing differential expression between the *in planta* and *in vitro* samples. The combination of proteogenomic and transcriptomic analyses has enabled the validation and fine-tuning of the majority of *de novo* predicted gene models. Several novel genes were identified via manual annotation of RNA-seq data. We have previously demonstrated that the genome is manipulable via Agrobacterium-mediated transformation which means that the functions of potential effector genes can be readily investigated. Collectively these data form a valuable resource from which a short list of effector candidates was derived and genes involved in the pathogenicity mechanisms of Didymellaceae fungi against their legume hosts were predicted.

633. Two G protein-coupled receptors, GprC and GprD, regulate density-dependent development in *Aspergillus flavus*. Katharyn J. Affeldt, Nancy P. Keller. University of Wisconsin-Madison, Madison, WI.

Aspergillus flavus is an opportunistic pathogen of several plant hosts, including maize. This interaction is mediated in part by oxygenated polyunsaturated acids, or oxylipins, that are produced by both the fungus and the plant host. Although much has been learned about the synthesis of these oxylipins, how the fungus perceives them remains unknown. We hypothesize that G protein-coupled receptors (GPCR) are responsible for receiving and transducing oxylipin signals in *A. flavus*. We have deleted and overexpressed two GPCRs, *gprC* and *gprD*, and found that they are important in regulating density-dependent development, which is thought to involve oxylipin signaling. Specifically, depletion of both *gprC* and *gprD* locks the fungus into a low-density state, even when grown at high density. Furthermore, this mutant is unable to respond to spent medium of a wild type high-density culture. Inoculation of these mutants on corn kernels will ask whether GprC and GprD are important for pathogenicity, and heterologous expression of GprC and GprD in *Saccharomyces cerevisiae* is being used to address questions concerning direct ligand-receptor activation.

634. Characterization of genes encoding putative secreted proteins during pathogenesis in *Magnaporthe oryzae*. Seongbeom Kim, Kaeun Kim, Sook-Young Park, Jaeyoung Choi, Junhyun Jeon, Yong-Hwan Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea.

The repertoire of secreted proteins defines the nature of interactions between microbe and host at the molecular level. Thus, cataloging and characterizing the list of secreted proteins from a given pathogen is a pivotal step in understanding molecular mechanisms of pathogenesis. Unlike bacterial and Oomycete pathogens, however, only a limited number of secreted proteins has been identified and analyzed in plant pathogenic fungi. Here we set out to identify and characterize new secreted proteins in the rice blast fungus. SingalP program predicted a total 1,885 genes encoding secreted proteins in *M. oryzae*. We prioritized 15 genes, *MoSPE1* to *MoSPE15*, with T-DNA mutants available for in-depth analysis. To reveal their roles in pathogenicity, gene deletion mutants were generated and characterized their functionality. Deletion of *MoSPE1* rendered the fungus non-pathogenic, while deletion of *MoSPE3*, *MoSPE6*, and *MoSPE15* resulted in reduced virulence. Rice sheath inoculation of D*Mospe1* and D*Mospe15* showed that defects in pathogenicity could be attributed to the inability to grow inside plant tissues, suggesting their implication in interaction with rice. In addition, the two genes were indeed up-regulated during invasive growth in rice. Proteins encoded by *MoSPE1*, *MoSPE6* and *MoSPE15* were capable of being secreted in yeast secretion trap system. We believe that our work would reveal novel function of secreted proteins, providing new insight into fungal pathogenesis.

635. The biosynthesis of oxalate is entirely dependent on oxaloacetate acetylhydrolase in *Sclerotinia sclerotiorum* . X. Liang[1], D. Liberti[2], M. Li[3], Y.-T. Kim[4], R. Wilson[1], J. Rollins[1]. 1) Plant Pathology Department, University of Florida, 1453 Fifield Hall, Gainesville, FL, 32611-0608; 2) Nunhems Netherlands BV, PO Box 4005, Haelen 6080 AA, Netherlands; 3) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 4) Environmental Biotechnology Research Centre, 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea.

Sclerotinia sclerotiorum (Lib.) *de Bary* is one of the most devastating necrotrophic fungal plant pathogens worldwide and its successful infection involves the accumulation of oxalate (up to 10 mM) in plant tissues. Oxaloacetate acetylhydrolase (EC 3.7.1.1), catalyzing the hydrolytic cleavage of oxaloacetate to form acetate and oxalate, has been shown to be the key enzyme catalyzing oxalate biogenesis in *Aspergillus niger*, *Botrytis cinerea* and *Cryphonectria parasitica*. To dissect the genetic regulation of oxalate biogenesis and pathogenesis of *S. sclerotiorum*, the *S. sclerotiorum* oxaloacetate acetylhydrolase gene *Ss-oah1* was functionally characterized. Previously we demonstrated that oxalate accumulation in *S. sclerotiorum* is under strong alkaline induction. Strikingly *Ss-oah1* gene expression is regulated in the same manner; neutral pH strongly induces the accumulation of *Ss-oah1* transcripts and this pH induction is completely suppressed in the *Ss-pac1* knock out mutant. *Ss-oah1* knock out mutants fail to accumulate oxalate in culture and during plant infection and these phenotypes are restored by complementation with the wild type gene. These data demonstrate that Ss-Oah1-catalyzed oxaloacetate hydrolysis is solely responsible for oxalate production in *S. sclerotiorum*. On all tested host plants, *Ss-oah1* knock out mutants are dramatically reduced in virulence and induce a strong host defense response. On leaves, *Ss-oah1* knock out mutants produce limited dark brown-green lesions compared with the spreading, necrotic, light brown lesions produced by the wild type. Host tissue bordering the lesion is clearly defined with a thin, dark zone and while the uninfected leaf tissue becomes yellow and senescent the colonized area often retains chlorophyll reminiscent of “green islands”. In sum, our experimental data establish the key function of oxaloacetate acetylhydrolase in oxalate biogenesis and pathogenesis in *S. sclerotiorum* and indicate that the oah1 oxalate minus mutant retains some aspects of virulence but cannot suppress host defense.

636. In vivo efficacy of antifungal treatment of Aspergillus terreus infections and the influence on host immune response in Galleria mellonella. Elisabeth Maurer[1], Neill Browne[2], Kevin Kavanagh[2], Cornelia Lass-Flörl[1], Ulrike Binder[1]. 1) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Tirol, Austria; 2) Medical Mycology Unit, Department of Biology, National Institute for Cellular Biotechnology, NUI Maynooth, Ireland.

Background Infections with Aspergillus (A.) terreus are of major concern, due to its high likelihood of dissemination and its intrinsic resistance to amphotericin B (amB). The reason for this resistance is not known yet and the exact mode of amB action is still not fully understood. Recently, three clinical isolates have been found to be amB susceptible in vitro. In order to investigate for differences in virulence of the respective isolates, and to test the amB efficacy in vivo, we used Galleria (G.) mellonella as an alternative model. Methods Virulence of amB resistant and amB susceptible A. terreus isolates was compared in the invertebrate model G. mellonella. Further, we performed in vivo infection studies with combined antifungal therapy, and additionally we investigated the potential effect of A. terreus infection and antifungal treatment on the G. mellonella immune system. Proteomic analysis of larval haemolymph, haemocyte counts and post-treatment infection studies were performed according to Kelly & Kavanagh 2011. Results Larval survival rates differed for the various isolates tested, resulting in highest mortality rate for one amB susceptible isolate. Increase in survival was seen for all tested strains, when larvae where treated with voriconazole. Treatment with amB only showed success in the groups infected with amB susceptible strains. Antifungal administration in larvae resulted in an increased number of circulating haemocytes. Proteomic studies showed different protein expression of a number of proteins which have immune function. Pre-treatment of larvae with different antifungals also increased their resistance to Staphylococcus (S.) aureus infection, indicating a general ability of antifungals to prime the insect's immune system.

637. Recognition and response to non self in Podospora anserina: a model of the fungal immune system. Marina Lamacchia, Annick Breton, Asen Daskalov, Frédérique Ness, Muhammad Khalid Salamat, Martine Sicault-Sabourin, Sven Saupe, Mathieu Paoletti. Institut de Biologie et Génétique Cellulaire, UMR 5095 CNRS et Université Victor Segalen Bordeaux, Bordeaux, France.

Recognition and response to non self, whether conspecific (between individuals from the same species) or heterospecifc (individuals from another species) is essential to many aspects of life including development, symbiosis and protection against pathogens. However distinction between these modes of recognition and responses is somehow blurred and can overlap. For instance in plants and animals Pathogen Recognition Receptors (PRRs) can occasionally lead to auto-immune diseases in absence of pathogens. The NLR and NBS-LRR STAND proteins (a class of signal transduction proteins) are major PRRs in plants and animals, but these receptors remain largely unidentified in fungi. In Podospora anserina vegetative incompatibility (VI), a conspecific non self recognition process, leads to cell death and autophagy. VI is determined by interaction of het-c, encoding a glycolipid transfer protein, with members of the hnwd gene family encoding for STAND proteins. hnwd gene family members display the hallmarks of PRR encoding genes, including fast evolution promoting production of a repertoire of receptors and ability to initiate a cell death reaction. het-c is also showing signs of fast evolution. We hypothesized that these genes are involved in pathogen recognition and that recognition of heterospecific non self would initiate a response similar to the VI reaction. In this context, VI can be considered as an autoimmune disease. We undertook the task of deciphering the response of P. anserina to heterospecific non self, focusing our efforts on the description of the cellular response and the identification of fungal PRRs. We show that P. anserina's responses to another fungal species (Epicoccum nigrum), or to bacteria such as Serratia entomophila or Pseudomonas putida largely overlap the VI response at all levels investigated so far, including cellular morphology and cytology, requirement of autophagy and induction of the expression of a set of genes. We also provide evidence that het-c encoding the GLTP contributes to the response to non self and argue that this protein may be targeted by pathogen's effectors. We develop efforts to identify PRRs involved in the initiation of these responses.

638. Increased late blight resistance in HIGS potato lines targeting a *P. infestans* gene. N. Temme, C. Blumenhagen, A. Schwarzer, L. Weimer, K. Prenzler, T. Sauter, M. Pflugmacher, D. Stahl. KWS SAAT AG, Einbeck, Germany.

Worldwide potato harvests are strongly diminished due the late blight disease caused by the oomycete *Phytophthora infestans*. The fungus-like eukaryote and its interaction with its host plants has been extensively investigated during the last decades whereas diverse research projects focus on its infection processes. *P. infestans* colonizes potato as well as tomato plants and thereby differentiates haustoria. Those barriers between host cells and invading pathogens are capable for exchange of nutrients, minerals but also of macromolecule like RNA molecules as shown for haustoria of parasitic plants. In oomycetes the exchange of effector protein from the pathogen to its host has been demonstrated. The movement of RNAi signals was shown in the interaction of parasites with their host plants and can be used to target not only plant genes but also genes of plant invading organism in a mechanism called host-induced gene silencing (HIGS). This technique has been applied for gene silencing in plant parasites as well as in nematodes and fungi. In oomycetes the RNA silencing is used as a standard method to characterize genes either by transient or by stable gene silencing and enzymes of the RNAi machinery have been identified. However, no efficient HIGS of oomycetes could be observed so far. We defined a *P. infestans* gene expressed during diverse developmental and infection stages of the oomycete as a HIGS target and could show that *in planta* expression of a HIGS hairpin construct targeting this particular gene in transgenic potato lines can be employed for late blight control. Our results present the appropriate processing of transformed HIGS hairpin constructs to siRNAs, their efficient function to silence the specific target gene sequence as shown in the reporter gene assays and subsequently reduced infection levels and diminished disease spreading on those transgenic HIGS potato lines in the field.

639. WITHDRAWN

Population and Evolutionary Genetics

640. Fertility in *Aspergillus fumigatus* and the identification of an additional 'supermater' pair. Céline M. O'Gorman[1], Sameira S. Swilaiman[1], Janyce A. Sugui[2], Kyung J. Kwon-Chung[2], Paul S. Dyer[1]. 1) School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; 2) Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

Aspergillus fumigatus is an opportunistic human pathogen that causes a range of allergic and invasive diseases in severely immunocompromised individuals, with a very high mortality rate typically in excess of 50%. A functional sexual cycle was discovered in 2009 and a highly fertile 'supermater' pair, AFB62 and AfIR928, was later identified from a collection of 50 isolates. Here we describe the results of a larger, worldwide fertility screen and present an additional 'supermater' pair. A set of 126 clinical and environmental *A. fumigatus* isolates were crossed against two Irish reference strains of each mating type. A subset of the eight most-fertile strains was then tested in all pairwise combinations. The pairing of isolates 47-169 x 47-154 had consistently high

mating efficiency and outcrossing ability after four weeks, therefore it was chosen as an additional 'supermater' pair for community use in mating projects. It is important to have alternative tester strains to allow for unexpected mating differences when crossing isolates of diverse genetic origins. This is because factors such as heterokaryon incompatibility (*het*) loci and single nucleotide polymorphisms, can considerably influence sexual compatibility. The worldwide fertility screen found that approximately 85% of isolates are sexually fertile, indicating that sexual reproduction should be possible in nature when suitable environments are present. Next, the plasticity of sexual crossing conditions was tested, to determine whether they could be manipulated to increase fertility in crosses involving low-fertility strains of interest. A range of environmental and growth conditions were examined, including incubation temperature, CO_2 level, and oatmeal agar type. Fertility levels were significantly affected by certain parameters. Work is ongoing to integrate these factors to further optimize fertility in the 'supermater' pairs.

641. Understanding the dynamic plant pathogen Ramularia collo-cygni at both the sequence and field level. James Fountaine, Peter Hoebe, Maciej Kaczmarek, Marta Piotrowska, Neil Havis. Crop and Soils Systems Research, Scotland's Rural College, Edinburgh, Scotland, United Kingdom.

The fungus Ramularia collo-cygni is the major biotic agent involved in Ramularia Leaf Spot (RLS). The fungus produces necrotic lesions on leaves, primarily after flowering takes place in the host plant. Despite being initially reported on crops in Italy in the late 19th Century RLS only became an economic pathogen of barley in the late 20th Century. The geographical spread of the disease now covers much of Europe, North and South America and New Zealand. Research in the last decade, using molecular tools, has helped elucidate the life cycle of the fungus and has indicated a seed borne stage. These tools have also allowed detailed testing of spring barley archive samples, which has revealed a significant increase in pathogen levels since the 1990's. The pathogen appears to develop rapid resistance in the field to fungicides and mutations conferring resistance have also been detected in the archive samples. Ramularia collo-cygni is currently classified as a member of the Mycosphaerella genera and sequence data derived within our group suggests a genetic similarity between R. collo-cygni, Mycosphaerella graminicola and M. fijiensis. These sequences focus primarily on the genes associated with the target sites for fungicides, such as Beta tubulin, Cytochrome b, Succinate dehydrogenase and eburicol 14a-demethylase (CYP51) genes. This presentation will demonstrate our current knowledge of this fungal pathogen and highlight the newly obtained genome and transcriptomic data generated by the combined approach of illumiina/solexa and Roche/454 sequencing. This combined approach has enabled the assembly of a complete genome sequence. The finished assembled genome of R. collo-cygni is 30.2 Mb and is currently to be found in 355 contigs. The complete annotation of this genome is currently underway using the FGENESH 2.6 software to generate first consensus gene calls. This approach will allow for comparative genome analysis in related genomes which will help to address the biology of R. collo-cygni in areas such as pathogenicity, population genetics and fungicide resistance. These advances should enable a greater understanding of the complex relationship between the fungus and host plant and furthermore, assist in the development of environmentally sound strategies to control this increasingly important disease of barley production systems.

642. Comparing germination dynamics of conidia and ascospores from natural isolates of *Neurospora crassa*. Kolea C.K. Zimmerman[1], Dan Levitis[2], Anne Pringle[1]. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Max Planck Institute for Demographic Research, Rostock, Germany.

Many organisms experience high mortality during the first stages of growth. This is especially relevant in fungi because of the large ratio between propagules produced and the number of those propagules that germinate and grow into a mature mycelium. Furthermore, in many organisms, asexually reproduced progeny have higher survival rates compared to sexually reproduced progeny. We have developed a high throughput pipeline using flow cytometry to analyze spore germination dynamics and have applied this pipeline to study the germination differences between asexual and sexual spores. Specifically, we applied this pipeline to study variation in germination of *Neurospora crassa* conidia and ascospores from 32 natural isolates and crosses among these isolates, respectively. Using publicly available sequence data, we computed all pairwise genetic distances between the 32 strains and crossed strains of varying genetic distance from each other to determine the effects of parent relatedness on ascospore viability. We found that viability of asexual spores is higher than viability of sexual spores in most cases and there is no clear linear relationship between genetic relatedness and ascospore viability. Future work will include experiments to evaluate the ability of sexual recombination to purge deleterious mutations. The data from these experiments will help us quantify the relative costs and benefits of asexual vs. sexual reproduction in *Neurospora crassa* and inform evolutionary theories on the evolution of sex.

643. Cryptococcus gattii and the origins of outbreaks. Tien Bui[1], Anna Foley[1], Leona Campbell[1], Patrick Brunner[2], Bruce McDonald[2], Dee Carter[1]. 1) School of Molecular Bioscience, University of Sydney, NSW 2006, Australia; 2) Institute of Integrative Biology, ETH, 8092 Zurich, Switzerland.

Cryptococcus gattii and its sibling species *C. neoformans* cause cryptococosis in humans and a range of animal. Strains within these species fall into a number of distinct molecular genotypes, and these vary in their ecology, geographic distribution, and various virulence-associated phenotypes. In *C. gattii*, molecular type VGI is found worldwide, usually in warmer regions, and causes sporadic infection in apparently healthy people and animals. VGII is more restricted in distribution, and while it also causes sporadic infection it is responsible for significant outbreaks that have expanded its geographic range into temperate areas. VGIII infections occur predominantly in immunocompromised hosts in the southern California region, and cases of VGIV infection are so far restricted to southern Africa, with a single case from India. Our interests lie in understanding the ecology and evolution of *C. gattii* in the environment, and how these relate to its ability to cause infection and outbreaks of disease. We have found the level of sexual recombination varies by molecular type, and that while in general the *C. gattii* population structure is sexual, this varies by genotype and in VGII is punctuated by periodic, clonal lineages. Here we refine our analysis using extended MLST data, haplotype networks and coalesence theory. We find the level of diversity among global VGI and VGII genotypes is highly constrained and comparable to some recently evolved plant pathogens, while the more geographically restricted VGIV genotype is substantially more diverse. Outbreak VGII clones are highly derived with an apparent history of expansion and extinction events. Evidence for purifying selection occurs at the master regulator of mating type for VGII, suggesting recombination is important in the generation of outbreak lineages.

644. Evolution of the mating type locus in the species within and closely related to the pathogenic *Cryptococcus* species complex. Sheng Sun, Josh Granek, Joseph Heitman. Molecular Genetics & Microbiology, Duke University, Durham, NC.

Cryptococcus amylolentus is the most closely related sister species to the pathogenic *Cryptococcus* species complex that includes the common human pathogenic fungi *Cryptococcus neoformans* and *Cryptococcus gattii*. We recently reported that *C. amylolentus* has a tetrapolar mating system, in which mating type is determined by two unlinked mating type (*MAT*) loci (A and B) that are located on different chromosomes. This is in stark contrast to the mating systems in the species within the pathogenic *Cryptococcus* species complex, where all the species are bipolar and have a contiguous large (>100 kb) *MAT* locus. Thus, analyzing the tetrapolar *MAT* loci of *C. amylolentus* could provide insights into how the derived bipolar MAT locus in the pathogenic *Cryptococcus* species complex evolved. In this study, we first provide a fully detailed characterization of both alleles for each of the two *C. amylolentus* *MAT* loci, illustrating expansion of the A *MAT* locus in *C. amylolentus*, as well as the chromosomal rearrangements between the alleles from the opposite

mating types. Additionally, by analyzing meiotic progeny of *C. amylolentus*, we found evidences that both *MAT* loci are linked to their respective centromeres. Using both genetics and genomics techniques, we further narrowed down the candidate centromeric regions to be located within 150 and 100 kb from the A and B *MAT* loci, respectively. Furthermore, genome comparison between *C. neoformans* and *C. amylolentus* showed that the majority of centromeres in *C. neoformans* are flanked by sequences from different chromosomes in *C. amylolentus*, indicating that ectopic recombination within centromeric regions may frequently lead to chromosomal translocations. We propose a model in which the large bipolar *MAT* locus that is present in the pathogenic *Cryptococcus* species complex originated through ectopic recombination in the centromeres (possibly mediated by common repetitive sequences present in the centromeric regions). This process brought together the two *MAT* loci of the ancestral tetrapolar mating system onto the same chromosome, and subsequent chromosomal rearrangements (inversions and transpositions) resulted in the current state of the *MAT* locus seen in the derived bipolar pathogenic *Cryptococcus* species.

645. Evolutionary history and genetic diversity of *Exobasidium* sp., the cause of an emerging disease of blueberry. Marin Brewer, Ashley Turner. Department of Plant Pathology, University of Georgia, Athens, GA.

Emerging fungal diseases, usually the result of pathogen introductions, the evolution of virulent races, or adaptation to new niches, are an increasing threat. Exobasidium fruit and leaf spot of blueberry has rapidly increased in incidence in the southeastern USA over the past two years. We took a phylogenetic approach to understand the evolutionary history of this fungus. We sequenced the LSU-rDNA region from nine isolates collected from fruit or leaf spots of *Vaccinium* spp. from Georgia and North Carolina. Additionally, sequences from GenBank with high similarity to the emerging parasite and from *Exobasidium* spp. parasitizing other *Vaccinium* spp. in North America were obtained. The sequences were assembled, aligned and subjected to phylogenetic analyses. Results indicated that *Exobasidium* sp. from blueberry in the southeastern USA is unique and distinct from *Exobasidium* sp. that causes a leaf spot on lowbush blueberry in the northeastern USA and Canada. Both species, however, are genetically different from other *Exobasidium* spp. that cause diseases on cranberry and blueberry, and from *E. vaccinii* from *V. vitis-idaea*. Results also suggested that within the Southeast the parasite is not genetically differentiated based on blueberry host species or cultivar, host tissue (fruit or leaf), or geographic region. To further investigate diversity and population structure of the parasite in the Southeast we sequenced ITS for 80 isolates from diverse host species, cultivars, and locations. We obtained 75 unique sequences, which is an extremely high level of diversity and is unexpected for any fungus let alone one causing an emerging disease. The high diversity indicates that the fungus causing Exobasidium fruit and leaf spot in the Southeast is not an evolutionarily young species and that the recent increase in incidence is not a result of increased aggressiveness or a recent host switch. Analyses of genetic differentiation of the ITS sequences confirm our findings with LSU-rDNA that isolates within the Southeast are not differentiated by host species or cultivar, host tissue, or geographic region, suggesting that a single population is causing this disease of blueberry across the Southeast. We hypothesize that an environmental change is responsible for the recent emergence of this disease.

646. Microsatellite markers reveal population structure and genetic diversity in the blueberry pathogen *Monilinia vaccinii-corymbosi*. Kathleen M Burchhardt, Marc A Cubeta. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

The ascomycete *Monilinia vaccinii-corymbosi* (Mvc) is a widespread fungal pathogen of blueberry (*Vaccinium* spp.) in North America. Both asexual and sexual spore production are required within a season for the fungus to complete its life cycle. Overwintered infected fruit (mummies) produce apothecia that release aerially dispersed ascospores which infect newly emerging blueberry shoots, resulting in blighting of infected tissues followed by production of conidia. Insect pollinators deposit conidia on flowers that infect the ovary through the gynoecial pathway, leading to fruit mummification. The primary objective of our research was to use population genetics-based approaches to examine genetic diversity, structure, and gene flow among populations of Mvc throughout the United States. A total of 437 samples from 18 blueberry fields in 10 states (one field in GA, MA, ME, MI, MS, NJ, NY, OR, and WA and 9 fields in NC) were analyzed with 10 microsatellite markers. Population genetic analyses supported population structure and high intraspecific genetic diversity, with 203 unique multilocus haplotypes (MLHs) identified from the samples. However, there were differences in genetic diversity and population structure based on locality and host species. Low genetic diversity and selfing were suggested based on analysis of samples of infected shoots or fruit collected from rabbiteye (*V. virgatum*) varieties in MS, GA, and five fields in NC. Only three unique MLHs were identified from analyzing the 141 samples collected from the seven fields, with two of the unique MLHs detected within four and five of the fields, respectively. At least 12 unique MLHs were detected within all other fields except OR, with all MLHs being exclusive to their field of origin. Samples from the 10 fields were collected from either infected shoots of rabbiteye, northern highbush (*V. corymbosum*), or southern highbush (*V. corymbosum x V. darrowii*), or from infected fruit of northern highbush or lowbush (*V. angustifolium*). Analysis of molecular variance and the software STRUCTURE supported significant genetic differentiation among these fields, indicating restricted gene flow. The majority of microsatellite markers were in linkage equilibrium within the fields, suggesting random mating. Future research will examine the potential for host specialization of isolates of Mvc.

647. Genetic diversity of Australian *Pyrenophora tritici-repentis* isolates using microsatellites. Caroline Moffat, Pao Theen See, Rick Dolling, Richard Oliver. Department of Environment & Agriculture, Curtin University, Perth, WA, Australia.

Pyrenophora tritici-repentis, the causal agent of tan spot of wheat, is an economically significant necrotrophic fungal pathogen. In Australia, tan spot is the most damaging wheat disease, resulting in yield losses of $212 million per annum. The disease was first recorded in Australia in the 1950s, some ten years after it was initially reported on wheat in the USA. Here, we examine the genetic diversity of a collection of Australian *P. tritici-repentis* isolates using microsatellites. We discuss relatedness and structure, and consider the findings in the broader context of biogeography.

648. WITHDRAWN

649. Fungal community composition analysis by Internal Transcribed Spacer (ITS) sequencing using Illumina MiSeq. Robin A. Ohm[1], Julien Tremblay[1], Kanwar Singh[1], Feng Chen[1], Claude Murat[4], Matthias Hess[1,2,3], Francis Martin[4], Susannah G. Tringe[1], Igor V. Grigoriev[1]. 1) US DOE Joint Genome Institute, Walnut Creek, CA., USA; 2) Systems Biology & Applied Microbial Genomics Laboratory, Washington State University, USA; 3) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory; 4) Lab of Excellence ARBRE, Tree-Microbes Interactions Department, INRA, Nancy, France.

Fungal species identification and community surveys relied for a long time on Internal Transcribed Spacer (ITS) sequencing using the Sanger platform. Later, 454 (Roche) pyrosequencing was used for the same purpose, capturing shorter ITS1 or ITS2 fragments, or more recently the entire ITS region using longer 454 XLR reads. The Illumina sequencing platform has now largely surpassed 454 in terms of read quantity and quality (e.g., HiSeq2000 yields of up to 600 Gb in a single run) but the length of produced reads (up to 150 bp in HiSeq2000) is insufficient for ITS analysis. Illumina's newly-introduced MiSeq sequencing platform can produce paired-end 250 base reads in a single day run, which, when combined, would cover most of either ITS1 or ITS2 regions.

At the US DOE Joint Genome Institute we tested the Illumina MiSeq platform for the analysis of fungal community composition in forest soil and cow rumen, and we developed a workflow for the subsequent data analysis. We surveyed fungal populations in these environments by targeting the ITS2 region. These amplicons were sequenced with an Illumina MiSeq instrument from both 5' and 3' ends with a 2x250 bases sequencing configuration. This was followed by in silico assembly using their shared overlapping part, where possible. The UNITE database of fungal ITS sequences was used as a reference database to classify the sequenced amplicons. As a classification method, both a naive Bayesian classifier (from the Ribosomal Database Project) and BLAST are explored. Our results suggest that the fungal population surveys on MiSeq successfully recapture known biological results and should provide a useful tool for fungal community characterization.

650. Estimation of genetic diversity of Ramularia collo-cygni populations using nuclear SSR markers to infer its potential to adapt to environmental changes. Marta Piotrowska[1], Fiona Burnett[1], Peter Hoebe[1], Richard Ennos[2], James Fountaine[1]. 1) Crop and Soil Research Group, Scotland's Rural College, Edinburgh, EH9 3JG, United Kingdom; 2) Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, EH9 3JT, United Kingdom.

Ramularia collo-cygni (Rcc) is a fungal pathogen of barley (Hordeum vulgare) but it can also infect other cereal crops such as wheat (Triticum aestivum), rye (Secale cereale) and oats (Avena sativa). Its economic impact has increased in the last two decades, when Rcc started to have an economic impact on grower's yields. Rcc has been present as a major barley pathogen in Scotland, since 1998. Quinone outside Inhibitor (QoI) fungicides were widely used to control the disease, but between 2001/2002 the first resistant strains appeared. Presently Succinate Dehydrogenase Inhibitors (SDHIs) are widely used and recommended as one of the most effective fungicide treatments against Rcc and currently all of the available data suggests that Rcc is still sensitive to all SDHI fungicides. However, Rcc has presently been exposed to SDHI fungicides for a number of growing seasons and the risk of fungicide resistance development is probably high. In this study we use newly designed SSR markers to describe the diversity of Rcc populations at field scale and understand its ability to adapt to environmental changes (i.e. fungicide applications). Using SSR markers we aim to obtain information about the distribution of genetic variation within and between Rcc populations and predict if clonal and/or sexual reproduction is taking place. Populations that are characterised by sexual or mixed reproduction systems over the growing season, may have higher adaptive potential than clonal populations, and thus could develop fungicide resistance more quickly. To study genetic variability in Rcc populations we developed 12 SSR markers and initially tested 10 isolates from 7 locations across the world: Austria, Switzerland, Czech Republic, Denmark, France, Great Britain and New Zealand. Eleven variable pentanucleotide repeat loci have been chosen for further testing. Further analysis was performed on a Scottish site, where 60 isolates were hierarchically sampled, and a Czech site where 30 isolates were sampled. Preliminary data collected from 10 isolates sampled worldwide indicates variability among Rcc populations that can not be explained by its geographical location alone.

651. Alkaloid genotype profiling of tall fescue endophytes to determine influence of ancestral progenitors. J.E. Takach, C.A. Young. Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

Epichloid endophytes, comprised of *Epichloë* and asexual *Neotyphodium* species, associate with cool-season grasses such as the agronomically important forage tall fescue (*Lolium arundinaceum* syn *Festuca arundinacea*). This mutualistic symbiosis provides the plant host with protection from animal and insect herbivory through the production of multiple classes of bioactive alkaloids (ergot alkaloids, indole-diterpenes, lolines, and peramine) by the endophyte partner. Many *Neotyphodium* species, including the endophytes present in tall fescue (*N. coenophialum*, *Festuca arundinacea* taxonomic groups FaTG-2 and FaTG-3) arise from interspecific hybridization events and contain genomic information from multiple ancestral progenitor species. As such, hybrid *Neotyphodium* species are capable of producing multiple classes of alkaloids and can contain multiple copies of the loci from required for alkaloid production. Significant genetic and chemotypic diversity has been reported for tall fescue endophytes but few studies have assessed this diversity at a population level. The incidence and diversity of tall fescue endophytes present in extant tall fescue seed collections was evaluated using PCR-based genotype profiling of seed from a set of 97 tall fescue accessions obtained from the Germplasm Resource Information Network (GRIN). A total of 71 endophyte-infected accessions were identified from both Continental (summer-active) and Mediterranean (summer-dormant) tall fescue germplasm. Genotype profiles from the GRIN tall fescue collection were compared to previously characterized tall fescue endophytes in order to predict the species and probable chemotype. Variation based on presence and absence of genes within the loci required for each alkaloid indicated likely chemotypic diversity among and between species. The copy number of selected alkaloid genes was determined by sequence analysis of PCR amplicons. The ancestral progenitor origins of mating type and alkaloid genes were inferred from phylogenetic analyses of partial gene sequences. These results support prior evidence that multiple alkaloid gene copies are the result of inheritance, not post-hybridization gene duplication, and suggest that multiple independent hybridization events have occurred during the evolutionary life history of tall fescue endophytes.

652. Evolution of the pan-secretome among lineages of *Magnaporthe oryzae* attacking different host-plants. E. Fournier[1], E. Ortega-Abboud[1,2], L. Mallet[3,4], H. Chiapello[3,5], C. Guérin[3], F. Rodolphe[3], A. Gendrault[3], J. Kreplak[4], J. Amselem[4], M-H. Lebrun[6], T. Kroj[1], D. Tharreau[2]. 1) INRA, BGPI lab, INRA, Montpellier, cedex 5, France; 2) CIRAD, BGP lab, TA 54K, 34398 Montpellier; 3) INRA, MIG lab, 78352 Jouy-en-Josas, France; 4) INRA, URGI lab, 78026 Versailles, France; 5) INRA, BIA lab, 31326 Castanet-Tolosan, France; 6) INRA, BIOGER lab, 78850 Thiverval-Grignon, France.

Over the past decade, considerable advances have been made in the understanding of the role of fungal effectors, and especially small secreted proteins (SSPs), in the infectious process. NGS technologies offer powerful tools to study, at the genomic scale, how deep are SSPs involved in the adaptation of fungal populations to different host plants. We addressed this question in the plant pathogenic fungus Magnaporthe oryzae, the agent of blast on rice and other Poaceae. This species encompasses isolated genetic lineages specifically attacking different hosts. In the GEMO project, we sequenced eight strains of M. oryzae representing different genetic groups pathogenic of different species of Poacees (5 strains attacking rice Oryza sativa, 1 attacking wheat Triticum sp., 1 attacking foxtail millet Setaria sp., 1 attacking finger millet Eleusine sp.), and one strain of the sister species M. grisea (attacking fonio millet Digitaria sp). The nine genomes have been sequenced using NGS technologies (454 and Solexa/Illumina) and assembled by the Genoscope (Evry, France). We included the public reference strain of M. oryzae 70-15 in our analyses. Gene annotation and orthology predictions have been carried out. We also annotated transposable elements and assessed the amount of horizontal transfers. Here we present the characterization of the repertoires of SSPs in the nine genomes, established using classical predictors of peptide signals (SignalP), transmembrane domains (TMHMM), GPI anchors (PrediGPI) and subcellular location assignment (TargetP). These lists were then curated using two complementary approaches: systematic tBlastn searches of the SSP predicted in each genome against the nine genomic databases of the project (including its own), and gene mining through the RNAseq analysis of the in planta transcriptome of one of the strain. We will compare these lists with orthology predictions to analyze the core-secretome and the dynamics of gains/losses/duplications of SSPs in the different lineages. We will also address the question of co-localization of SSPs with transposable elements. Finally we will search for signatures of adaptive evolution in SSPs.

653. Exploiting the high evolutionary potential of *Leptosphaeria maculans* minimises severity of blackleg disease of canola. Steve J. Marcroft[1], Angela P.

Van de Wouw[2], Barbara J. Howlett[2]. 1) Marcroft Grains Pathology, Grains Innovation Park, Horsham, 3400, Vic., Australia; 2) School of Botany, the University of Melbourne, 3010, Vic., Australia.

Blackleg caused by *Leptosphaeria maculans*, is the most important disease of *Brassica napus* (canola) worldwide. Field populations of this sexually outcrossing fungus rapidly adapt to selection pressure from extensive sowing of varieties with major gene resistance and can 'overcome' resistance. This high evolutionary potential of the fungus is reflected in its genome structure. Effector genes are embedded in AT-rich, gene- poor regions with transposable elements that have been degenerated by Repeat Induced Point (RIP) mutations. Thus effectors are easily gained, lost or inactivated. For the last decade we have monitored virulence of blackleg populations and disease severity of varieties in field trials across Australia. In 2003 after two seasons of extensive sowing, blackleg resistance of a set of varieties 'broke down' in the Eyre Peninsula, South Australia, causing 90% yield losses and withdrawal of these varieties from sale. By 2005, virulence of populations towards these varieties declined appreciably. Thus the blackleg-canola interaction behaves in a 'Boom and Bust' manner. Analysis of isolates collected before and after the resistance breakdown showed that deletions, RIP mutations and amino acid substitutions accounted for rapid evolution of four linked effectors, including the avirulence gene complementary to the resistance gene that had been overcome. After this resistance breakdown, Eyre Peninsula farmers sowed varieties with a different source of resistance. However in November 2011 significant levels of disease in trial sites and commercial paddocks were observed, which led to a warning in February this year that these varieties should not be sown. Growers heeded this advice and sowed varieties with different resistance sources. Our prediction of a resistance 'breakdown' was vindicated, as this variety had high disease levels in field trials on Eyre Peninsula, but not in other canola-growing regions. Commercial crops of other varieties on Eyre Peninsula had only low levels of disease. By sowing other varieties, not only have farmers have been saved $20 million (based on conservative estimates of area sown, predicted yield loss and current canola prices), but seed companies have been able to sell the 'at risk' varieties in other canola-growing regions, where resistance breakdown was not predicted.

654. Experimental demonstration of Crozier's paradox in fungi. Eric Bastiaans, Alfons J.M. Debets, Duur K. Aanen. Plant Science Group, Wageningen University, Wageningen, Netherlands.

Kin selection can favour cooperation between individuals. This requires assortment between genetically related individuals and genetic kin recognition is the predominant means to achieve this. However, Crozier realised that the diversity of kin-recognition alleles necessary for kin recognition, observed in many social organisms, poses a paradox: common alleles will receive more cooperation than rare alleles, and therefore increase in frequency, thus eroding genetic kin recognition diversity. We provide experimental evidence for Crozier's theoretical prediction using somatic fusion between fungal individuals (mycelia) as a model for cooperation. Using fusion mutants and incompatible strains, we first show that fitness is strongly correlated with the degree of fusion, which demonstrates that fusion between mycelia is mutually beneficial. We then experimentally demonstrate Crozier's prediction that positive frequency-dependent selection erodes kin-recognition diversity.

655. A completely unknown lifecycle in mushrooms: cyclical inbreeding and haplo-diploidy. Duur K. Aanen[1], Tim Möhlman[1], Eric Bastiaans[1], Bart Nieuwenhuis[1], Bertha Koopmanschap[1], Thomas W. Kuyper[2]. 1) Plant Science Group, Wageningen University, Wageningen, Netherlands; 2) Department of Soil Quality, Wageningen University, Wageningen, The Netherlands.

Mycena galericulata (Basidiomycota, Agaricales) occurs in two forms, a clampless with two-spored basidia and a clamped with four-spored basidia. It is generally accepted that the two-spored form is haploid asexual (apomictic), and the four-spored form sexual (dikaryotic and heterothallic). In order to study the interrelationship between both forms, we performed mating tests and phylogenetic and genetic analyses of a sample of both forms. Surprisingly, our results are inconsistent with any currently known life-cycle. While the four-spored form is heterothallic indeed, we show that the two-spored form is diploid, and produces diploid spores via intra-tetrad selfing. However, the absence of genetic differentiation between both forms, and the high degree of heterozygosity in the two-spored form, indicate that the two-spored form frequently arises from the four-spored. We hypothesise that the two-spored form can again give rise to four-spored forms. Consistent with this, we discovered that a small percentage of fruiting bodies has both two-spored and four-spored basidia.

656. Diversity and evolution of ABC proteins in basidiomycetes. Andriy Kovalchuk[1], Yong-Hwan Lee[1,2], David Hibbet[3], Fred O. Asiegbu[1]. 1) Department of Forest Sciences, University of Helsinki, Finland; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea; 3) Department of Biology, Clark University, Worcester MA 01610, USA.

ABC proteins constitute one of the largest families of proteins. They are implicated in a wide variety of cellular processes ranging from ribosome biogenesis to multidrug resistance. With the advance of fungal genomics, the number of known fungal ABC proteins increases rapidly, but the information on their biological functions remains scarce. In this work, we extended our previous analysis of fungal ABC proteins to include recently genome sequenced species of basidiomycetes. We performed an identification and initial cataloguing of ABC proteins from 23 new species representing 10 orders from within the class of Ascomycotina. To identify gene loci encoding ABC proteins in the fungal genomes, multiple tblastn and blastp searches against selected genomes were performed at the website of the Fungal Genomics Program of the Department of Energy Joint Genome Institute (JGI). Sequences of Coprinopsis cinerea ABC proteins representing all known subfamilies were used as queries. Phylogenetic analysis was performed with the program package MEGA5 using neighbor-joining, minimum evolution and maximum likelihood algorithms and bootstrapping with 500 replicates. ABC proteins of each species were separated into subfamilies by their comparison with S. cerevisiae, C. cinerea, C. neoformans and U. maydis proteins. Set of ABC proteins identified in basidiomycetes and ascomycetes were compared, and their common features and principal differences are discussed. Two groups of ABC proteins specific for basidiomycetes were identified. Results of the survey should contribute to a better understanding of evolution of ABC proteins in fungi and support further experimental work on their characterization.

657. Co-evolution and life cycle specialization of plant cell wall degrading enzymes in a hemibiotrophic pathogen. Patrick C. Brunner[1], Stefano F. F. Torriani[1], Daniel Croll[1], Eva H. Stukenbrock[2], Bruce A. McDonald[1]. 1) Integrative Biology, ETH Zurich, Zurich, Switzerland; 2) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

Co-evolution of species has long been recognized as a driving force in generating and maintaining biodiversity. Co-evolution is an ubiquitous phenomenon investigated in prey and predator, plant and herbivore, or mutualistic interrelationships. However, signatures of co-evolution are likely to be strongest in host-pathogen systems because of the strong selective pressures that each can exert directly on the other. While traditional studies mainly sought phenomenological evidence for co-evolution, more recent approaches look directly at the molecular/gene level. We hypothesized four main scenarios for host-pathogen co-evolution and predicted the corresponding genetic signatures. We combined comparative genomics, transcriptomics and selection analyses to investigate genes that are likely affected by co-evolution to assign them to one of these scenarios. Zymoseptoria tritici is an important fungal pathogen on wheat and has two closely related sister species Z. pseudotritici and Z. ardabiliae that infect wild grasses. This recently

emerged host-pathogen system provides a rare opportunity to investigate the dynamics of gene evolution by natural selection within and between species and on different hosts. Here, we focused on evolution of plant cell wall degrading enzymes (PCWDEs) secreted by the fungus. We found widespread differential transcription among different members of the same gene family, challenging the idea of functional redundancy and suggesting instead that specialized enzymatic activity occurs during different stages of the pathogen life-cycle. We also found that natural selection has significantly affected at least 19 of the 48 identified PCWDEs. The majority of genes showed signatures of purifying selection, typical for the scenario of conserved substrate optimization. However, six genes showed diversifying selection that could be attributed to either host adaptation or host evasion. This information can be used to determine which genes are the most appropriate targets for subsequent wet lab experimentation to elucidate enzymatic function during relevant phases of the pathogen life cycle.

658. Recombination landscape of the plant pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*). D. Croll, M. Lendenmann, E. Stewart, M. Zala, B.A. McDonald. ETH Zurich, Zurich, Switzerland.

Recombination is a fundamental process driving the evolution of genomes. The rate of recombination influences the level of genetic variation in populations and the efficacy of selection. Furthermore, heterogeneity in recombination rates along chromosomes shapes the genetic architecture of phenotypic traits. Hence, the evolution of virulence and other traits in pathogenic fungi critically depends on the rate of recombination. Despite the importance of recombination, the rate and homogeneity of recombination in fungal chromosomes is poorly understood. We analyzed 60 progeny from a controlled sexual cross between two isolates of the wheat pathogen *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*). We genotyped parental strains using whole-genome resequencing and we generated progeny genotypes by restriction site-associated DNA sequencing (RADseq). We obtained a total of 46'037 single nucleotide polymorphisms (SNP) segregating among the progeny. Based on the physical and genetic map locations of the markers, we showed that recombination rates were strongly heterogeneous along chromosomes and were influenced by gene density and GC content. We located multiple chromosomal hotspots of recombination that were interspersed by large segments of low recombination rates. Furthermore, we found that chromosomal regions that were enriched in SNP and indels showed lower recombination rates compared to less diverged regions. The local variation in recombination rates in *Z. tritici* may have significant effects on the evolutionary potential of different genomic compartments. Hence, heterogeneity in recombination rates may play an important role in the evolution of virulence.

659. The evolution of Sfp1 mediated, cell size control in Ascomycete fungi. Toni M. Delorey[1], Jenna M. Pfiffner[1], Sushmita Roy[2], Jay Konieczka[1], Dawn A. Thompson[1], Aviv Regev[1]. 1) Broad Institute, 7 Cambridge Center, Cambridge, MA 02139; 2) Wisconsin Institute for Discovery (WID), 330 N. Orchard St, Madison, WI 53715.

Divergence in gene regulation can play a major role in evolution. We used a phylogenetic framework to measure mRNA profiles in 15 yeast species and reconstruct the evolution of their modular regulatory programs. We found that modules diverge with phylogenetic distance, with prominent regulatory changes accompanying changes in lifestyle and ploidy. Gene paralogs have significantly contributed to this regulatory divergence. To explore the role of *trans* regulator duplication, we examined Sfp1, as gain or loss of the Sfp1 binding site underlied regulatory rewiring of carbon metabolism. In *S. cerevisiae*, Sfp1, a TOR target, activates transcription of "growth" genes. *S. cerevisiae,* sfp1D mutants have smaller cells and slower growth, suggesting that these phenotypes are intertwined. However, we show that duplication of SFP1 in other yeast species has resulted in sub- and neo-functionalization of regulatory programs controlling growth rate and cell size. In particular, in *S. castellii* , the two Sfp1 paralogs have subfunctionalized; one controls cell size while the other controls growth. Therefore, we hypothesize that Sfp1 regulation of ribosome biogenesis underlies growth rate while cell size is mediated by a different, unidentified function. To better understand Sfp1-mediated cell size control, we used a two-tiered analysis system of comparing gene expression and ChIP Seq data to distinguish indirect or direct Sfp1 targets. Expression programs and phenotypes of sfp1D mutants were analyzed in *S. cerevisiae*, *C. glabrata, S. castellii, K. lactis* and *S. pombe.* To identify putative cell size regulators, we examined differentially expressed orthologs in species where sfp1D mutants had a small size phenotype (*S. cerevisiae, C. glabrata* and one paralog of *S. castellii*), and excluded genes involved in ribosomal biogenesis and those differentially expressed genes in species where sfp1 mutants grew slower but had normal cell size (*K. lactis* and *S. pombe*).We found 17 overlapping orthologs including a promising candidate for cell size regulation; the *S. cerevisiae* ortholog, Ard1, involved in telomeric silencing, and cell cycle control. Finally, we found that Sfp1 binds to the SCH9 promoter in *S. cerevisiae* and *S. paradoxus*. Sch9 is a kinase and mutants have reduced cell size. From these findings, we present a novel model for cell size regulation.

660. Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta. Pierre Gladieux, David Kowbel, Christopher Hann-Soden, John Taylor. Department of Plant and Microbial Biology, University of California, Berkeley, CA.

Identifying the genes for ecologically relevant traits is a central challenge in empirical population genetics. Species distributed across strong environmental gradients are excellent models to discover and identify the genetic targets of local selection as they are more likely to experience spatially heterogeneous selection pressures leading to local adaptation of ecologically important traits. We studied the origin of ecological differentiation in N. discreta phylogenetic species 4 (PS4), a species with a broad latitudinal distribution. We Illumina-sequenced the complete genomes of 52 individuals representing 8 collections sites in Alaska, New Mexico, Washington, California, and Western Europe (average sequencing depth: 52X). Reads were mapped to the N. discreta PS4 reference genomes, and analyses were based on a final set of ca. 1.2 million high-quality SNPs. Phylogenetic analyses identified four well-supported clades. Papua New-Guinea individuals formed the most basal clade. Individuals from Alaska and Europe on the one hand, and from New Mexico on the other hand grouped into sister clades, and individuals from California were basal to these two clades. Individuals from Washington, sampled within the same site, grouped with either the New Mexico individuals, or the California individuals, indicating the coexistence in sympatry of two divergent populations. The observed pattern of population subdivision is being used as a reference to identify genes departing from the genome-wide background, and showing increased divergence consistent with divergent selective pressures, or decreased divergence consistent with gene-flow. Our findings emphasize the need to continue exploration to uncover divergent populations of Neurospora, and place N. discreta, along with N. crassa, among the handful of species that have the attributes to serve as outstanding evolutionary and ecological model organisms.

661. WITHDRAWN

662. Evolutionary genomics of NRPS gene clusters in *Beauveria* and its allies. J.-G. Han[1], J. Oh[3], M.-W. Hyun[2], B. Shrestha[1], G.-H. Sung[1]. 1) Mushroom Research Division, Rural Development Administration, Suwon 441-707, Republic of Korea; 2) College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea; 3) Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan 330-714, Republic of Korea.

Beauveria is an ascomycetous asexual genus that comprises of 12 species of insect pathogens and linked to its teleomorphic stage of *Cordyceps*. Among species of *Beauveria*, *Beauveria bassiana* is economically important for its use as biological control agent and produces several secondary metabolites such as beauvericin and bassianolide, which are the causal metabolites of entomopathogenicity. Genes involved in these secondary metabolites are including

nonribosomal peptide synthetase (NRPS) that are often physically clustered. To more understand the origins and evolution of these genes, we performed genome-wide comparative analyses among *B. bassiana* and its allied species including *B. pseudobassiana*, *B. sungii*, *Cordyceps militaris*, *C. pruinosa*, *Isaria tenuipes*, and *I. farinosa* after assembling these genomes. Initially, all the potential gene clusters for secondary metabolites were predicted using by antiSMASH. In *B. bassiana*, 16 NRPSs and 3 NRPS-PKS hybrid modules were estimated, while in *C. militaris*, 5 NRPSs and 3 hybrids were found. Interestingly, the presence/absence of NRPSs and PKSs provides a clue in generating their evolutionary hypotheses (e.g., horizontal gene transfer and gene fusion). For example, bassianolide synthetase were specifically found in the species referred to *Beauveria* with the syntenic conservation of genes encoding calreticulin, cyclophilin, DNA replication complex, IdgA domain protein, and phenol 2-monooxygenase. The inferred phylogeny based on the acyltransferase (AT) domain showed that ATs of beauvericin synthetase gene belong to two independent lineages, suggesting that beauvericin synthetase is a fusion gene which is formed from two previously separated genes. It is also suggested that bassianolide synthetase in *Beauveria* spp. are possibly acquired by horizontal gene transfer (HGT) from distantly related fungi.

663. Neurospora presents a model for the evolution of mating systems. Christopher Hann-Soden, Pierre Gladieux, John Taylor. Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

The study of plants and animals has yielded a host of theories for the evolution of outbreeding and selfing mating systems, yet these theories have yet to be extended to microbial organisms, where they might be more easily challenged. Within the genus Neurospora there is evidence of multiple shifts to selfing (homothallic) from outcrossing (heterothallic) ancestral states. However, the most well-studied clade, the members of which produce brightly colored macroconidia, contains no homothallic members, and there is only one known clade of aconidial heterothallic Neurospora from which homothallic Neurospora could have evolved (Nygren et al. 2011, Glass et al. 1990). We hypothesized that sampling of heterothallic Neurospora has been biased toward members that produce brightly colored macroconidia or perithecia in isolation, thereby ignoring aconidial heterothallic species. Collections of cryptic heterothallic Neurospora would allow comparison of the evolutionary forces acting on closely related pairs of selfing and outcrossing species. To this end, we have isolated 1 new strain of heterothallic Neurospora and 9 new strains of homothallic Neurospora from soil from two locations in California. Based on partial sequences of nik-1 (Nygren et al. 2011) we constructed a phylogeny of Neurospora including the new strains. Surprisingly, the new heterothallic Neurospora was found to be almost identical to the only other aconidial, heterothallic Neurospora individuals (Glass et al. 1990), despite over 2,300 km of separation. Seven of the eight sequenced homothallic strains were found to be most closely related to Neurospora novoguineensis, but the remaining strain was more closely related to the aconidial, heterothallic strains. These preliminary results suggest that additional sampling to obtain sufficiently large populations of related, aconidial heterothallic and homothallic Neurospora would facilitate studies of the transition from outbreeding to selfing as well as studies of the genetic basis of the development of macroscopic, brightly colored conidia. References, Glass, N. L., Metzenberg, R. L. & Raju, N. B. Experimental Mycology 14, 274-289 (1990). Nygren, K. et al. Molecular phylogenetics and evolution 59, 649-63 (2011).

664. Profiling conditionally dispensable chromosomes of the plant-pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*). Ronny Kellner[1], Veronika Schott[1], Stephan Poppe[1], Rachel Brem[2], Eva H. Stukenbrock[1]. 1) Max Planck Institute for Terrestrial Microbiology, Fungal Biodiversity, Karl-von-Frisch-Strasse 10, 35043, Marburg, Germany; 2) University of California, Department for Molecular Cell Biology, 176 Stanley Hall, Berkeley, CA 94720-3220, USA.

Conditionally dispensable chromosomes (cDCs) are common genomic features in many parasitic ascomycetes. The presence of cDCs entails a high amount of intraspecific genomic variation that is inherited in a non-Mendelian manner. Because genes located on cDCs have in some species been shown to play a role in pathogenicity cDCs may promote rapid adaptive evolution in response to host defenses. With up to eight cDCs the genome of the wheat pathogen *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*) contains by far the largest known proportion of dispensable elements among all ascomycetes. In comparison to the core chromosomes, cDCs of *Z. tritici* are smaller, have on average less and shorter genes with a lower GC content and a higher amount of paralogous sequences and repetitive elements. Hitherto, the functional relevance of cDCs for *Z. tritici* remains unclear.

In this study we elucidate the relevance of cDCs in *Z. tritici* by assessing expression profiles during in-planta and axenic growth. Because our RNAseq dataset covers both host and non-host interactions we broaden the perspective of our approach by insights into host-specific expression profiles. In order to verify the current genome annotation we mapped all RNAseq reads to the genome of *Z. tritici* and predicted gene transcripts. By combining our results with the latest genome annotation we set up a new transcript list, which was used in further analyses. We demonstrate an overall significantly lower transcription of genes located on cDCs relative to genes located on core chromosomes. In addition, cDCs encode several unique genes that are expressed under certain conditions. We identify duplicated genes using a blast approach and show differential gene expression between paralogs on cDCs and core chromosomes. To link the transcription of cDC genes to specific stages in the interaction of *Z. tritici* and wheat we focus on a gene family of three paralogs where two genes encode secreted proteins. We quantified their expression via qPCR at seven different time points of the interaction. In summary, our study suggests the functional relevance of single cDC genes and the relevance of cDCs for gene innovation and adaptive evolution in *Z. tritici*.

665. Take a walk on the wild side: evolutionary consequences of resistance to apple scab introgressed from a wild host. C. Lemaire[1], T. Leroy[1], M. de Gracia[2], C-E. Durel[2], M. Templeton[3], J. Bowen[3], V. Caffier[2], B. Le Cam[2]. 1) IRHS, University of Angers, Angers, France; 2) IRHS, INRA, Beaucouzé, France; 3) Plant & Food Research, Auckland, New Zealand.

Theories on emergence of pathogens are dominated by ecological hypotheses. For instance overcoming of a host resistance gene is often described as a consequence of the spreading of a mutant that invades resistant hosts from infected susceptible cultivars. However invasion of virulent populations from wild habitats are often neglected. Though taking into account the occurrence of a preexisting allopatric virulent population greatly impacts conclusions made about emergence of new virulence and the nature of barriers to reproduction with the avirulent population. Indeed under allopatric scenario, these barriers are not expected to be only adaptive. Here, we intended to decipher the evolutionary history of the European populations of the apple scab pathogen Venturia inaequalis enable to overcome the Rvi6 resistance gene introgressed in Malus x domestica from the crabapple Malus floribunda. Using microsatellite and sequence data in an Approximate Bayesian Calculation (ABC) framework, we demonstrate that virulence corresponding to a new resistance gene introgressed in agrosystems was previously present in the wild within a population that has diverged since several thousands years. We show that deployment of the corresponding resistance gene in agrosystems can then act as gateways for virulent populations to invade orchards. At last we show that secondary contact followed by mating between invading and resident population can reveal genetic incompatibilities (Dobzhansky-Müller Incompatibilities) accumulated during divergence between the two populations. These incompatibilities induce hybrid depression by negative epistasis. This study based on state-of-the-art tools of population genomics, phenotyping and genetic mapping is the first to demonstrate the occurrence of intrinsic post-zygotic barriers in pathogens revealed by a secondary contact. Overall, it points out the risk of generating new “hybrid” populations harbouring new pathogenic traits by introgression of resistance genes in agrosystems by breeders.

666. Genomic footprint of adaptive divergence in *Ophiostoma montium*, a fungal symbiont associated with the mountain pine beetle. J. F. Mao, B. Dhillon, C. Tsui, K. Ritland, R. Hamelin. Faculty of Forest Sciences, University of British Columbia, Vancouver, BC, Canada.

Ophiostoma montium is the most common fungus associated with the mountain pine beetle, the insect responsible for the destruction of 18 million ha of pine forests in Canada. In order to determine the evolutionary histories of *O. montium* populations, high-throughput genome sequencing was used to uncover the genetic changes that accompany divergence as lineages colonize different conifer hosts in different climatic regions. 36 fungal isolates from different hosts and different environments were sequenced. *De novo* genome assembly from one isolate was used as reference to call variants (922,000 SNPs and 126,000 Indels). Multiple analyses including whole genome variants, genetic distance, population structure and Identity by descent (IBD) identified three population lineages, corresponding to the various hosts and three geographic groups: US, Rocky, and North Canada (NC). Faster linkage disequilibria (LD) decays were observed in both Rocky and NC groups, indicating an increase in genomic recombination and/or high effective population size. Presence of genomic regions with high LD and negative Tajima's D was evidence that population size expansion (after bottleneck or a selection sweep) and/or purifying selection occurred in the NC group. Loci in the genome contributing to both host shift and climatic transition were also identified by multiple lineage-specific genomic scans for selection. Additionally, genome recombination events were recovered for lineages experiencing different demographic histories. Our study highlights the value of whole genome sequences both in evolutionary dynamics and genetics of plant pathogens.

667. Ecological context in symbioses: when is your enemy also your friend? Georgiana May[1], Paul Nelson[2]. 1) Dept Ecol, Evol, Behavior,#100, Univ Minnesota, St Paul, MN; 2) EEB graduate program University of Minnesota St. Paul MN.

Most plants are rife with fungal symbiotic partners with many of these having little apparent effect on the host's health and fitness. In this work, we explore the degree to which the outcome of interactions between an endophytic fungus, pathogen and plant host depend on ecological context. In particular, we ask whether interactions between the endophyte of maize, Fusarium verticilliodes, with the pathogen Ustilago maydis, depend on host resistance to the pathogen. In the case of a host susceptible to the pathogen, the two fungal species should meet frequently, and compete over host resources, potentially driving greater virulence to the host in one or the other fungal species. In the case of a host resistant to the pathogen, the endophyte might be a "bystander" to the pathogen, because the two meet too infrequently to drive their co-evolutionary interaction. We show evidence that the two fungal species have evolved stronger antagonistic interactions in maize susceptible to the pathogen, and further, that this might be associated with greater virulence by the pathogen. Results of modeling will also be presented from which we predict longer term evolutionary trajectories for this 3-way interaction.

668. Population shifts and mating-type heterokaryosis in *Aspergillus flavus*. Rodrigo A. Olarte[1], Bruce W. Horn[2], Carolyn J. Worthington[1], Rakhi Singh[1], Ignazio Carbone[1]. 1) Department of Plant Pathology, North Carolina State University, Raleigh, NC; 2) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA.

Aspergillus flavus is a heterothallic fungal pathogen of many economically important crops worldwide. We sampled *A. flavus* strains from a cornfield in Rocky Mount, North Carolina, USA. Plots were inoculated at tasselling with either *A. flavus* AF36 or NRRL 21882 (=Afla-Guard) nonaflatoxigenic biocontrol strains, both of which are mating type *MAT1-2*. Subsequently, aflatoxigenic strain NRRL 3357 (*MAT1-1*) was applied to all plots, including control plots not inoculated with biocontrol strains. Sclerotia were harvested from infected corn ears and ninety single-ascospore isolates were obtained from ascocarps originating from plots treated with AF36 and NRRL 21882. In addition, eighty *A. flavus* isolates were collected from soil one month after planting (before biocontrol application) and one year after biocontrol application, for a grand-total of 250 isolates. PCR amplification revealed grouping of isolates into three distinct mating-type classes: *MAT1-1*, *MAT1-2* and *MAT1-1*/*MAT1-2*. An overwhelming majority (54%) of isolates sampled prior to biocontrol treatments were heterokaryotic for mating type (*MAT1-1*/*MAT1-2*), but was shifted to only 9% of isolates from soil after biocontrol treatments; 39% of isolates obtained from ascospores were heterokaryotic, with the remaining comprising either *MAT1-1* or *MAT1-2*. Multilocus genotyping indicated that ascospores might have originated from Afla-Guard as a putative parent; there was no evidence of AF36 or NRRL 3357 in ascospores or in pre- or post-treatment soil samples, which may explain the genetic structure of the indigenous population. The vertical transmission of *MAT1-1*/*MAT1-2* to progeny ascospore isolates suggests that heterokaryosis can be maintained in subsequent generations. Furthermore, matings were performed to determine functionality of these *MAT1-1*/*MAT1-2* strains and all isolates tested were strictly functional as *MAT1-2*. Further characterization of heterokaryons and their frequency in *A. flavus* populations may be important in understanding the adaptation of these fungi to changing environmental conditions and could lead to better and more effective biocontrol strategies specific to a geographic region. Understanding population structure is the key to unlocking the secrets of a successful biocontrol strain.

669. Structural variation of trichothecene mycotoxins has resulted from multiple evolutionary processes in the fungal order *Hypocreales*. R. H. Proctor[1], A. M. Stanley[1], M. G. Malmierca[2], N. J. Alexander[1], S. Gutiérrez[2], S.P. McCormick[1]. 1) Bacterial Foodborne Pathogens and Mycology, USDA ARS NCAUR, Peoria, IL; 2) Universitary School of Agricultural Engineers, University of León, Ponferrada, Spain.

Trichothecenes are secondary metabolites produced by fungi in at least six genera of the order *Hypocreales*. These metabolites are of concern because they are toxic to humans and other animals and can accumulate in grain used for food and feed. They also contribute to plant pathogenesis of *Fusarium* and to biological control activity of *Trichoderma*. Although all trichothecenes share the same molecular skeleton, a tricyclic structure with a12,13-epoxide, different genera produce trichothecenes that differ in patterns of oxygenation and acylation. To investigate how such structural variation has evolved, we examined 1) variation in gene function and content in homologs of the trichothecene biosynthetic gene (*TRI*) cluster and 2) phylogenetic relationships of *TRI* genes among trichothecene-producing genera. The results suggest that the ancestral hypocrealean *TRI* cluster consisted of at least seven genes, including the enzyme-encoding genes *TRI4* and *TRI5* responsible for synthesis of the trichothecene skeleton, the regulatory genes *TRI6* and *TRI10*, and the transporter gene *TRI12*. Phylogenetic analyses indicate that oxygenation of carbon atom 4 (C-4), which occurs in all trichothecene-producing genera, likely arose when different genera acquired different C-4 hydroxylase genes: e.g. *TRI11b* in *Trichoderma* and *Myrothecium* and *TRI13* in *Fusarium*. In contrast, C-3 oxygenation, which occurs in only one genus, likely arose by a change in function of *TRI4*, a gene that exists in all genera. These results, and those from studies of *Fusarium* and *Trichoderma*, indicate that structural variation of trichothecenes has arisen by recruitment, changes in function, and deletion of *TRI* genes during evolution of the *Hypocreales*.

670. Evidence for birth-and-death evolution and horizontal transfer of the fumonisin mycotoxin biosynthetic gene cluster in *Fusarium*. R.H Proctor[1], F. Van Hove[2], A. Susca[3], G. Stea[3], M. Busman[1], T. van der Lee[4], C. Waalwijk[4], A. Moretti[3], T.J.. Ward[1]. 1) Bacterial Foodborne Pathogens and Mycology, USDA ARS NCAUR, Peoria, IL; 2) Earth and Life Science Institute, Université catholique de Louvain, Louvain, Belgium; 3) Institute of Sciences of Food Production, National Research Council, Bari, Italy; 4) Plant Research International B.V., Wageningen, The Netherlands.

In fungi, genes required for synthesis of secondary metabolites are often clustered. The *FUM* gene cluster is required for synthesis of fumonisins, a family of toxic secondary metabolites produced predominantly by species in the *Fusarium* (*Gibberella*) *fujikuroi* species complex (FFSC). Fumonisins are a health and agricultural concern because their consumption is epidemiologically associated with multiple diseases in humans and other animals. Among FFSC species, the *FUM* cluster is discontinuously distributed but uniform in gene order and orientation. In this study, we demonstrate that the *FUM* cluster exists in at least four different genomic contexts within the FFSC and that phylogenetic relationships derived from analyses of *FUM* cluster genes are correlated with genomic context, but are inconsistent with species relationships inferred from analyses of primary-metabolism genes. In addition, analyses of synonymous site divergence suggested that *FUM* cluster divergence predated divergence of the FFSC. These results are not consistent with trans-species evolution of ancestral cluster alleles or with interspecies hybridization, but suggest duplication of the cluster within an FFSC ancestor and subsequent loss and sorting of paralogous clusters in a manner consistent with the birth-and-death model of evolution previously described for multigene families. A model based on horizontal gene transfer (HGT) could also explain these observations, but seems unlikely because it requires independent transfer events from multiple unknown donors to multiple FFSC recipients. However, analyses of phylogenetic relationships and synonymous site divergence provided strong evidence that *F. oxysporum* strain FRC O-1890 acquired the *FUM* cluster via a relatively recent HGT event from *F. bulbicola* or a closely related species within the FFSC. These results indicate that, as with other secondary metabolite clusters, species phylogenies do not provide an adequate picture of the complex evolutionary history of the *FUM* cluster within *Fusarium*.

671. Chemotype predominance in *Fusarium graminearum* is not directly affected by the use of the fungicides trifloxystrobin and isopyrazam. Matias Pasquali, Tiphaine Dubos, Friederike Pogoda, Lucien Hoffmann, Marco Beyer. Environment and Agro-biotechnologies Department, CRP GABRIEL LIPPMANN, Belvaux, Luxembourg.

Mitochondrial respiration inhibitors are effective fungicides used to control wheat diseases in Europe. Since the beginning of the monitoring of chemotype diversity in wheat fields in Luxembourg, we are trying to identify factors involved in the shift of chemotype prevalence (nivalenol vs. 15ADON vs. 3ADON). In this study we investigated whether the use of fungicides belonging to the respiratory inhibitors complex II and III (that are used in wheat fields for treating other diseases) may play a role in selecting a specific *F. graminearum* chemotype. Strains from Luxembourg and from a world collection with isolation dates ranging from 1969 to 2011 were chemotyped by genetic means and then analysed for their sensitivity to trifloxystrobin (inhibitor of respiratory complex III) and isopyrazam (inhibitor of respiratory complex II) using a microplate in vitro test on conidia. The maximum level of inhibition which could be obtained by trifloxystrobin ranged from 14 to 65% for the 55 strains analyzed with no complete inhibition up to a concentration of 3mM. Fortyone isolates tested for their sensitivity towards isopyrazam were insensitive with the average rate of inhibition converging towards 28%. For both fungicides, EC50 values did not significantly depend on the chemotype, suggesting that these two fungicides do not exert a direct pressure on the selection

of chemotypes in *F. graminearum*. Our study also suggests that *F. graminearum* seems to be significantly insensitive to respiratory complex inhibitors (II and III). Molecular mechanisms involved in the insensitivity are under investigation.

672. Evolution of races within f.sp *lycopersici* of *Fusarium oxysporum*. BV. Chellappan, PM. Houterman, M. Rep, BJC. Cornelissen. Molecular Plant Pathology, University of Amsterdam, SILS, Science Park 904, 1090 GE Amsterdam, The Netherlands.

Three physiological races (1, 2 and 3) of *Fusarium oxysporum* f.sp *lycopersici* (Fol) have been identified based on their inability to infect tomato cultivars carrying Fol resistance genes (*I*, *I-2* or *I-3*, respectively). We wished to unravel the molecular mechanisms underlying the evolution of Fol races. It is generally assumed that race 2 evolved from race 1 by loss of *AVR1* and that race 3 evolved from race 2 by a point mutation in *AVR2*, thus overcoming *I* and *I-2* mediated resistance, respectively. We have sequenced a genomic region of approximately 100 kb containing *AVR1* in race 1 isolate Fol004 and compared it to the sequenced genome of race 2 isolate Fol4287. A genomic fragment of 30.5 kb containing *AVR1* was found to be missing in Fol4287. Further analysis suggests that race 2 evolved from race 1 by deletion of this 30.5 kb fragment, most likely due to recombination between helitrons bordering the fragment. A worldwide collection of Fol isolates was subjected to PCR analysis of the *AVR1* genomic region, including the two bordering helitrons. The results suggest that, based on the deletion event that led to loss of *AVR1*, Fol isolates can be divided into distinct lineages that coincide with their geographical origin. Our results also suggest that transposable elements played a major role in the evolution of races within f.sp *lycopersici* of *Fusarium oxysporum*.

673. Detection of Mitochondrial DNA Heteroplasmy in the progeny of crossed genetically divergent isolates of Arbuscular Mycorrhizal Fungi. Maryam Nadimi, Ivan de la Providencia, Gabriela Rodriguez, Denis Beaudet, Moahmed Hijri. IRBV, Biological Sciences Dep., University of Montreal, Montreal, QC, Canada.

Nonself fusion and nuclear genetic exchange has been documented in arbuscular mycorrhizal fungi (AMF) particularly in *Glomus irregulare*, which is a common and widespread species. However, mitochondrial transmission accompanying nonself fusion of genetically divergent isolates remains unknown. We developed a series of crossing experiments between different isolates of *G. irregulare*, harboring genetically divergent mitochondrial DNA (mtDNA) haplotypes. We tested the hypothesis that heteroplasmy (i.e. mixture of genetically different mtDNA in a common cytoplasm) occurs in the progenies of the crossed isolates. Three isolates of geographically distant locations were used to investigate nonself fusions and mtDNA transmission in the progeny. To be able to trace the mtDNA haplotypes, we sequenced two mtDNAs of two *G. irregulare* isolates (DAOM-240415 and DAOM-234328) additional to the current available isolate DAOM-197198. We developed isolate-specific markers in variable regions of intergenic mtDNAs (*cox3-rnl*) of these isolates. Three crossing combinations in pre-symbiotic and symbiotic phases were performed. Interestingly, nonself fusion frequency was low and was usually associated with irregular shape and aborted spores, although normal spores were also observed. Ten progeny spores per crossing combination were genotyped using isolate-specific markers. We showed the evidence that nonself fusion occurs between isolates originated from different continents both in pre-symbiotic and symbiotic phases. Genotyping patterns of individual spores from the progenies clearly showed the presence of markers of the two parental mtDNA haplotypes. Our results demonstrated the occurrence of mtDNA heteroplasmy in the progeny of crossed isolates. This raises the questions whether mtDNA heteroplasmy is transient or persistent in AMF? What are their consequences in evolution of AMF? Are there any conflicts of the presence of mtDNA heteroplasmy within an individual? Further studies on vegetative compatibility and incompatibility and putative sex machinery in AMF will provide new information to explore and solve these questions and thereby advance our understanding of the evolution of AMF.

674. Evolution of mode of infection in the rice blast fungus and allied species. Ning Zhang[1], Shuang Zhao[1], Jing Luo[1], Guohong Cai[1], Debashish Bhattacharya[2], Bradley Hillman[1]. 1) Plant Biology and Pathology, Rutgers Univ, New Brunswick, NJ; 2) Ecology, Evolution and Natural Resources, Rutgers Univ, New Brunswick, NJ.

The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as *Pyricularia oryzae* (Magnaporthe oryzae, rice blast fungus), *Magnaporthiopsis poae* (Magnaporthe poae, summer patch pathogen of turf grasses), and *Gaeumannomyces graminis* (take-all fungus of various cereals and grasses), which are popular model organisms in fungal biology and host-pathogen interaction studies. Despite their ecological and economic importance, the phylogenetic relationships among the constituent species remain ambiguous due to the lack of convincing morphological characters and paucity of molecular data for the majority of the non-model species in the family. In this study, our multilocus phylogeny suggests that both *Magnaporthe* and *Gaeumannomyces* are polyphyletic genera. Therefore, a new genus, *Magnaporthiopsis* is proposed based on phylogeny and morphology. The phylogeny also provides insights into fungal biology and pathogenesis. *Pyricularia oryzae* formed a basal clade, while *Magnaporthiopsis poae* and *Magnaporthiopsis rhizophila* formed another well-supported clade with *Magnaporthiopsis incrustans* (G. incrustans), *G. graminis* and *Nakataea sigmoidea* (Magnaporthe salvinii). The basal species infects both root and aerial parts of plant host, while the aerial infection capacity seems to be lost in the taxa of the latter clade. The study indicates that anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among these taxa. In addition, we performed genome sequencing for 6 species in Magnaporthaceae: *Magnaporthiopsis rhizophila, Magnaporthiopsis incrustans, Harpophora maydis, Nakataea sigmoidea, Ophioceras dolichostomum,* and *Pseudohalonectria lignicola*, in order to conduct phylogenomic and comparative genome analyses for both pathogenic and non-pathogenic members of this family.

675. WITHDRAWN

676. Population genomic analysis reveals a complex evolutionary history of Neurospora tetrasperma. Padraic Corcoran[1], Fen Chen[2], Martin Lascoux[1], Peixiang Ni[2], Hanna Johanesson[1]. 1) Uppsala University, Uppsala, Sweden; 2) BGI, Hong Kong, Hong Kong.

Fungal population genomics as a field of inquiry has seen a rapid growth in the recent years, with ability to sequence the genomes of multiple strains of fungi sampled from many populations. These studies have aimed at utilizing a population genomic approach to understanding the evolutionary forces that have had the greatest effect in shaping the genomes of fungal species. In this study, we extend the use of population genomics to help understand the evolutionary history of Neurospora tetrasperma. Neurospora tetrasperma has been the focus of much research in recent years, with most effort devoted to the study of its predominantly non-recombining mating type chromosomes. Here we present results of analysis on the whole genome resequencing of 86 homokaryotic strains of N. tetrasperma, sampled from locations in England, New Zealand and Louisiana, USA, together with one strain each of the close heterothallic species N. hispaniola and N. sitophila. These genomes were sequenced to a mean depth of between 25 to 30X coverage. Phylogenetic and population structure analysis of the genomewide SNP data produced identified that the sequenced strains of N. tetrasperma belong to 5 previously recognised lineages of N. tetrasperma. Comparisons of the multiple N. tetrasperma genomes with the N. sitophila, N. hispaniola and N. crassa genomes confirmed previous observations on introgression, but also revealed signatures of introgression between the English population of N. tetrasperma and N. hispaniola. Furthermore, comparisons between the genomes of the two homokaryons isolated from each heterokaryon revealed a large number of

differences across all chromosomes in the UK population of N. tetrasperma, while the other lineages show much fewer heteroallelic sites. Analysis of the mating type chromosome reveals considerable variation in the sizes of putative regions of suppressed recombination in the 5 lineages of N. tetrasperma investigated. We also present findings on the genome wide patterns of polymorphism within populations and divergence between populations, with the aim of dissecting which regions of the genome have been recently acted on by natural selection. The initial results gained in this study are showing that multiple N. tetrasperma populations have complex histories that have left strong signatures in their genomes.

677. Rapid evolution of female-biased genes: a novel example from the eukaryotic model organism *Neurospora crassa*. Hanna Johannesson, Carrie Whittle. Evolutionary Biology, Uppsala University, Uppsala, Sweden.

In animals and plants, sex-biased gene expression plays a major role in gene evolution. In particular, reproductive genes with male-biased expression tend to exhibit rapid protein evolution and reduced codon bias as compared to female-biased or unbiased genes. Minimal data are available for fungi. Here, we demonstrate that sex-biased expression is associated with gene evolution in the filamentous fungus *Neurospora crassa*, but in contrast to animals and plants, the rapid evolution occurs for female-biased genes. Based on analyses of >25,000 expressed sequence tags (ESTs) from male (conidial), female (protoperithecial) and vegetative (mycelial) tissues, we show that reproductive genes with female-biased expression exhibit faster protein evolution and reduced optimal codon usage than male-biased genes and vegetative genes. Furthermore, our data suggest that female-biased genes are also more apt to experience selective sweeps. The sex-biased expression effects are observable at the species and population level. We argue that the rapid molecular evolution of female-biased genes is best explained by sexual selection via female-female competition, but could also result from mate-choice and/or directional natural selection.

678. Fungal Community Dynamics During Biomass Degradation in the Cow Rumen Determined by ITS Sequencing. Hailan Piao[1,2], Julien Tremblay[3], Robin Ohm[3], Kanwar Singh[3], Fernanda Haffner[4], Stefan Bauer[4], David Culley[2], Kenneth Bruno[2], Kerrie Barry[3], Feng Chen[3], Scott Baker[2,5], Roderick Mackie[6], Susannah Tringe[3], Igor Grigoriev[3], Matthias Hess[1,2,3,5*]. 1) Washington State University, Richland, WA; 2) DOE Pacific Northwest National Laboratory, Richland, WA; 3) DOE Joint Genome Institute, Walnut Creek, CA; 4) Energy Biosciences Institute, UC Berkeley, Berkeley, CA; 5) Environmental Molecular Science Laboratory, Richland, WA; 6) University of Illinois, Urbana-Champaign, IL.

The microbial community that inhabits the cow rumen is composed of Archaea, Bacteria and Eukarya and is well known for its biomass-degrading ability. In order to understand this ecosystem at the whole-systems level it is important to monitor the dynamics of the individual community members. Sequencing of the 16S rRNA gene has been used intensively to obtain insights into the ecology of the prokaryotic fraction of the microbial rumen community. To obtain insights into the ecology of the fungal fraction of the rumen community and its dynamics during biomass-degradation, we amplified the ITS2 region from fungi that colonized corn stover and switchgrass during rumen incubation. Amplicons were generated from rumen-incubated switchgrass and corn stover and rumen fluid at six different time points and from two different host animals. Sequencing on Illumina's MiSeq platform resulted in a total of 10,675,384 sequences with an average read length of ~240bp amounting to a total of >2.6 Gbp of sequence information. Succeeding sequence analysis revealed a fungal community of low complexity, with two phyla as the dominant players. Members of the phylum Neocallimastigomycota were absent on the pre-incubated biomass and appeared to colonize both corn stover and switchgrass throughout the incubation process. Members of the phylum Ascomycota were less dominant (<1%), but seemed to play a role in the later phases of the biomass-degradation process. In summary, results presented here demonstrate that rumen fungi consistently colonize lignocellulosic substrates and may play a role in the degradation of recalcitrant biomass.

679. A Systematic and Genomic Description of *Undulatus ophiodiicola*, Formerly Referred to as Chrysosporium ophiodiicola, an Emerging Fungal Pathogen of Snakes. Mana Ohkura[1,2], James Hughes-Hallet[2], Jeremy Worley[2], Robert R. Fitak[2], Jennifer H. Wisecaver[2], Daniel DeBlasio[2], Andrew Gloss[2], Christopher Brownlee[2], Erik Hanschen[2], Noëlle Bittner[2], M. Alejandra Mandel[1], Brenda Love[3], Jenny Fisher[3], A. Elizabeth Arnold[1], Marc J. Orbach[1]. 1) Division of Plant Pathology, University of AZ, Tucson, AZ; 2) NSF IGERT Genomics Program, University of AZ, Tucson, AZ; 3) Pennsylvania State Animal Diagnostic Laboratory, The Pennsylvania State University, University Park, PA.

In the order Onygenales there are many well-studied mammalian fungal pathogens, including both dermatophytes and systemic pathogens, such as *Trichophyton rubrum*, *Cocciodioides* spp., and *Histoplasma capsulatum*. Recently, a new species in the Onygenales was described, *C. ophiodiicola*, an emerging pathogen of snakes. However, members of the genus *Chrysosporium* produce aleuriospores and arthrospores with limited characteristics making it difficult to define morphological boundaries for the genus and to distinguish it from other genera with similar spore types. As a result, the genus is polyphyletic containing species that are not that closely related. Here, we report the characterization of a fungus with a nearly identical ITS sequence to *C. ophiodiicola* from two diseased snakes in Pennsylvania and present a molecular and morphological revision of the phylogenetic placement of *C. ophiodiicola*. Molecular analysis reveals that this fungus belongs to the same clade as *C. ophiodiicola*, but that their placement in the genus *Chrysosporium* is inaccurate. Thus, we propose the description of a new genus, *Undulatus*, to accommodate our fungus as well as *C. ophiodiicola*. In addition, we will present recent results from our comparative analysis of the Undulatus ophiodiicola genome and its pathogenic and non-pathogenic relatives within the Onygenales. Comparisons of genome statistics, gene family expansions and contractions, unique KEGG categories, and secondary metabolite clusters will be presented. To relate the molecular results with biochemical characteristics, the ability of *U. ophiodiicola* and related fungi to grow on different substrates was assessed using Biolog plates. We compared both the *U. ophiodiicola* genome and growth results to develop hypotheses on how variability in pathogenicity and host specialization evolved among these related taxa.

680. Pair-wise linkage disequilibrium decay among linked loci suggests meiotic recombination in natural populations of *Sclerotinia sclerotiorum*. Renuka Nilmini Attanayake[1], Weidong Chen[2]. 1) Washington State University, Johnson Hall 303, pullman, WA. 99164; 2) USDA ARS Grain Legume genetics and physiology research unit, Pullman WA 99164.

Both clonal and recombining population structures have been reported in *Sclerotinia sclerotiorum* populations around the world. Association of independent and putatively unlinked markers indicates clonal population structure, whereas random association of the markers suggests recombination and outcrossing. However, high mutation rates of markers used for inference, like certain microsatellite markers, could interfere and compromise the inferences of recombination and outcrossing. To test if the recombination is due to outcrossing or mutation, we used 12 microsatellite loci distributed over four chromosomes to genotype 230 isolates sampled from seven populations in the USA and China from a variety of crops. All the isolates produced single alleles for each of the loci tested, indicating all the isolates were homokaryotic for the microsatellite loci in consideration. Pair-wise linkage disequilibrium (LD) tests (Hedrick's D, Fishers exact test and IA) between physically linked loci showed relationships ranging from linked to random association with increasing distance between loci on three chromosomes. For the three loci on chromosome four, LD decay with increasing physical distance between loci was found in six of the seven populations. Likewise, LD decay was found for the three loci on chromosome six in four of the seven populations, and also for

the loci on chromosome five in two of the populations. The reduced pair-wise linkage disequilibrium with increasing distance cannot be attributed to mutation alone, and thus the high intrachromosomal recombination is most likely due to meiotic recombination following outcross in these populations. Recombination hot spots and cold spots were detected. Mating type loci in 59 isolates of two populations were genotyped using PCR with allele-specific primers. About 40% of the isolates showed both *MAT1-1* and *MAT 1-2* idiomorphs, as expected for a homothallic species. However, the remaining 60% of the isolates had only the *MAT1-2* idiomorph as detected by the allele-specific PCR. Although the nature of the absence of the *MAT1-1* idiomorph remains to be determined, the results showed variations in mating type alleles in natural populations, suggesting that some of the isolates may not be truly homothallic.

681. Population genomics of *Suillus brevipes*. Sara Branco, John Taylor, Tom Bruns. Plant and Microbial Biology, University of California, Berkeley, CA.
Environmental heterogeneity may result in divergent selection, which in turn may lead to local adaptation resulting in populations showing habitat-based discontinuous variation. Very little is known on the patterns of adaptive divergence and local adaptation or underlying selective forces in ectomycorrhizal fungal populations. We are studying the population genomics of *Suillus brevipes* (Peck) Kuntze, an ectomycorrhizal fungus associated with pine trees. Our goals are 1) to test the existence of barriers to gene flow across populations, and 2) detect genomic regions exhibiting selection sweeps. We sequenced the whole genomes of 30 *S. brevipes* individuals from two populations, one from a coastal Bishop pine forest in Mendocino Co (CA) and another from a high altitude Lodgepole pine forest in Yosemite National Park (CA). One isolate was selected as the reference strain and its genome was assembled de novo through a collaboration with the Joint Genome Institute. All other individuals were aligned to this reference and single nucleotide polymorphisms detected across populations. These markers are being used to find regions with evidence of positive natural selection, including possible islands of introgression, increased rates of non-synonymous substitutions, accelerated rates of divergence, and gene duplications. Results from our study will make a significant contribution for understanding patterns of neutral and functional variation as well as gene flow and selection in ectomycorrhizal fungi.

682. Ugly, understudied and undertreated: population genomics of the most common human fungal pathogen - the dermatophyte Trichophyton rubrum. M. Gajdeczka, W. Li, J. Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.
Trichophyton rubrum causes athlete's foot, nail infections and ringworm in about twenty percent of the general population. Despite an annotated genome and hundreds of clinical isolates, the global genomic diversity and population biology of T. rubrum are not well understood. Additionally, T. rubrum cannot be crossed, genetic manipulation is difficult, and animal infections with T. rubrum are acute and highly inflammatory (rather than chronic and relatively mild as in humans). These factors have made it difficult to study virulence in T. rubrum and have limited the development of effective chemotherapies. This study will test three hypotheses: (1) clonal reproduction and limited dispersal have resulted in undiscovered local sequence variation and linkage disequilibrium (LD); (2) observed variation supports an out-of-Africa isolation-by-distance migration model; and (3) patterns of sequence diversity in sex- and virulence-associated genomic regions support the significant evolutionary roles of reproductive clonality and close interaction with humans. Worldwide isolates appear reproductively clonal and remarkably genetically monomorphic. Five common MLST markers reveal no variation among 50 European, Asian and North American strains. We Sanger sequenced 7.5 kb of non-coding intergenic regions in ten T. rubrum isolates collected over a span of ten years. These sequences revealed no polymorphisms useful for differentiating strains. Lastly, VNTR genotyping by our lab and other groups revealed two broad geographic types, though these are based on a few highly variable loci subject to homoplasy. We are assembling whole genome sequences of 32 geographically and clinically diverse strains to characterize sequence diversity more completely. The genome obtained by the Broad Institute will be used as a reference. We will obtain measures of diversity, polymorphism, LD, population differentiation and divergence. These measures will be used to compare isolates genome-wide, determine population structure and infer evolutionary and demographic forces contributing to observed patterns of variation. Locus-specific markers will be developed based on standing variation to genotype additional strains. Furthermore, we will test for variants associated with hypothesized virulence mechanisms involved in chronic T. rubrum infection.

683. Poppr: an R package for genetic analysis of populations with mixed reproduction. Zhian N. Kamvar[1], Niklaus J. Grünwald[1,2]. 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) USDA-ARS Horticultural Research Laboratory, Corvallis, OR.
Analysis of populations with mixed reproductive systems, including a blend of sexual and clonal reproduction, remains a challenge. We developed an R package implementing existing approaches for analysis of mixed populations. R is a multi-platform, open source, statistical environment that has gained popularity over the past few years. While there are a plethora of packages in R that perform population genetics analyses, many standard analysis methods for populations with mixed modes of reproduction remain hard to accomplish. Poppr aims at providing functions to facilitate rapid analyses of this data, particularly including methods for analysis of recombination (index of association), clone-censored analysis of full datasets in a hierarchical manner over all levels of sampling, genotypic diversity analyses, and distance analyses. As implemented, poppr requires minimal commands with convenient summary functions while providing compelling graphics. Unlike many platform dependent, standalone programs, poppr can be used for batch processing of data including all kinds of population genetic data (dominant/codominant; microsatellites, AFLP, SNPs). Poppr is available as a beta release for testing upon request and continues to be improved.

684. The heterothallic fungus *Cercospora beticola* contains fragments of both mating type genes. Melvin D. Bolton[1], Zhaohui Liu[2]. 1) USDA - ARS, Fargo, ND; 2) North Dakota State University, Department of Plant Pathology, Fargo, ND.
In most heterothallic Ascomycota, the ability to reproduce sexually is determined by a single mating type locus (*MAT1*) represented by two idiomorphs known as *MAT1-1* and *MAT1-2* that are required to control nonself-recognition and mating of compatible partners. To investigate the *MAT1* locus in the heterothallic fungus *Cercospora beticola*, we performed Southern analyses of both mating types using each mating type gene (*MAT1-1-1* and *MAT1-2-1*) as a probe. Surprisingly, several bands of similar size were observed in both mating types using either probe, suggesting that multiple loci contain sequences of both *MAT* genes in the *C. beticola* genome. We screened a BAC library derived from a *MAT1-2* isolate using both *MAT* gene probes to identify clones containing *MAT* gene sequences. Sequence analysis of four BAC clones confirmed that one BAC contained the true *MAT1-2* idiomorph, while the other three contained fragments of both *MAT* genes in close proximity (8 to 1,075 bp) to each other. To investigate whether this holds true in the opposite mating type, we sequenced the identical regions in a *MAT1-1* isolate. Sequence analysis confirmed that the *MAT1-1* isolate contained one full *MAT1-1* idiomorph as well as additional regions that contained fragments of both *MAT* genes. In all cases, introns normally present in *MAT* genes were not present in the *MAT1-1-1* or *MAT1-2-1* gene fragments in either mating type. Although heterothallism has been suggested to be the ancestral state in the Dothideomycetes, the identification of fragments of both mating types in a single isolate suggests that homothallism is the ancestral state in *C. beticola*.

685. Population structure from mountain to coast of two Lophodermium endophytes; a case study comparing rare and common fungal species in pine needles. Ryoko Oono[1,2], Laurel Koch[1], A. Betsy Arnold[3], Georgiana May[4], François Lutzoni[1], Ignazio Carbone[2]. 1) Department of Biology, Duke University,

Durham, NC; 2) Department of Plant Pathology, North Carolina State University, Raleigh NC; 3) School of Plant Sciences, University of Arizona, Tucson AZ; 4) Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN.

Many foliar fungal endophytes are transferred horizontally among plant hosts and exhibit various degrees of host specificity. For example, endophytic Lophodermium species (Rhytismatales) are commonly found in needles of certain pine species but infrequently in broad leaves of angiosperm hosts. Such endophytes may have populations or subspecies that are genetically structured according to their host preference, climate regimes or geographic ranges, which might be associated with increased fitness for plants hosting specific mutualistic endophytic fungi. Hence, we set out to explore the population structure of Lophodermium spp. within mature foliage of loblolly (Pinus taeda) and Virginia pine (Pinus virginiana) in southeastern U.S., covering host ranges from the mountains of Appalachia to the coasts of the Atlantic. We analyzed the nuclear ribosomal internal transcribed spacer (ITS1/5.8S/ITS2) and the intragenic spacer (IGS) regions as well as three protein-coding genes (actin, calmodulin, chitin synthase I) of the isolated Lophodermium strains. These in-depth population genetic analyses of endophyte species provide insight into the capacity of multilocus genetic markers that resolve on different evolutionary time scales to capture sub-species level structure and to identify criteria for species delimitation. This multilocus analysis identified a rare cryptic species, which possibly explains the high genetic diversity of the Lophodermium spp. Genetic and geographic distance were correlated in the rare cryptic species, but not in the common species. We found no evidence for host species preference for the cryptic species vs. the common species. Our findings suggest that genetic exchange and recombination can be limited by dispersal if the species is rare.

686. Fungal pathogen and endophyte genetics within the context of forest community dynamics. M.-S. Benitez[1], M. H. Hersh[2], L. Becker[1], R. Vilgalys[3], J. S. Clark[1,3]. 1) Nicholas School of the Environment, Duke University, Durham, NC; 2) Department of Biology, Eastern Michigan University, Ypsilanti, MI; 3) Department of Biology, Duke University, Durham, NC.

Fungal pathogens play important roles in forest community dynamics, particularly through negative-density dependent regulation. Negative-density dependence regulation is hypothesized to be regulated by the presence of host-specific pathogens. Studies on forest pathogens, however, indicate the predominance of generalist seedling pathogens, capable of infecting more than one host species. To understand the mechanisms through which "generalist" pathogens contribute to forest-community dynamics we conducted extensive surveys of seedling pathogens in temperate hardwood forests of the eastern U.S.A. Species in the genera *Colletotrichum* and *Ilyonectria* were among the most commonly isolated and recovered amplicon sequence from seedlings of multiple host species showing disease symptoms. Further, co-infection by both *Colletotrichum* and *Ilyonectria* species decreases host survival, as quantified by posterior model probabilities. To investigate molecular mechanisms associated with multi-host generalism and co-infection, and to determine whether these "generalist" pathogens are distinct species or species-complexes, the genomes of three common species in our dataset (e.g. *C. fioriniae*, *C. gloesporoides* and *Ilyonectria europea*) were sequenced. The largest genome of the three belonged to *Ilyonectria* at 63.66 Mb, which also contained the highest number (22,250) of genes. The smallest genome belonged to *C. fioriniae* with 50.04 Mb and 15,777 genes. Genome size and number of predicted genes appears expanded, confirming their role as seedling pathogens. For instance, three out of four polysaccharide lyase (PL) enzyme domains found in fungal genomes, are enriched in these three species. PL enzymes are relevant in plant pathogenicity since they may contribute to initial stages of host penetration. The genome sequence of these fungal groups will serve as a reference set for population level studies to address host-specificity and local adaptation within our isolate database.

687. Discovery of Sexual Reproduction in the Black Aspergilli. Heather L. Darbyshir[1], Peter JI. van de Vondervoort[2], Paul S. Dyer[1]. 1) School of Biology, University of Nottingham, Nottingham, NG7 2RD United Kingdom; 2) DSM Biotechnology Center, PO Box 1, 2600 MA Delft, The Netherlands.

The black aspergilli are members of the genus *Aspergillus* that are typically characterized by the production of dark or black asexual conidia (classified as section *Nigri*). The group includes *Aspergillus niger*, which is of particular industrial importance because of its safe use status and ability to produce a wide range of enzymes and organic acids. All members of the black aspergilli have previously only been known to reproduce by asexual means. However, as a result of combined molecular and cultural experimental studies it can now be revealed that at least one member of the black aspergilli, *Aspergillus sclerotiicarbonarius*, is able to complete a sexual cycle. Wild type isolates of *A. sclerotiicarbonarius* were found to retain the ability to form sclerotia, structures associated with both dormancy and sexual reproduction, and strains of complementary MAT1-1 and MAT1-2 could be identified based on the presence of mating-type genes. Crossing strains of opposite mating type, and an extended period of incubation, resulted in the production of sclerotia containing multiple ascocarps, with asci and viable ascospores, within the matrix of a sclerotium. This is consistent with past studies of phylogenetically related species in the *Aspergillus* section *Flavi* (teleomorph genus *Petromyces*). Progeny analysis is being undertaken based on data arising from comparative genome sequencing of parental isolates, mating-type distribution and phylogenetic analysis. The discovery of a heterothallic sexual cycle in *A. sclerotiicarbonarius* provides insights into the evolution of asexuality in the black aspergilli. It is hoped that ongoing molecular genetic studies into the early sexual morphogenesis may provide an insight into the regulation of sexual reproduction in the black aspergilli.

688. Culture-based survey of soil fungi from bat hibernacula. Jeffrey M. Lorch[1], Daniel L. Lindner[2], Andrea Gargas[3], Laura K. Muller[4], Andrew M. Minnis[2], David S. Blehert[4]. 1) University of Wisconsin - Madison, Madison, WI, USA; 2) US Forest Service, Northern Research Station, Center for Forest Mycology Research, One Gifford Pinchot Drive, Madison, WI, USA; 3) Symbiology LLC, Middleton, WI, 53562, USA; 4) US Geological Survey - National Wildlife Health Center, Madison, WI, USA.

Bat white-nose syndrome (WNS), a fungal disease now spreading in eastern North America, is causing unprecedented mortality among hibernating bats. To investigate fungal communities present in bat hibernacula, we identified culturable fungi present in soil samples from 24 bat hibernation sites in the eastern United States. Isolates were characterized by sequencing regions of ribosomal DNA (internal transcribed spacer and partial intergenic spacer). We isolated *Geomyces destructans* from soil samples collected in hibernacula within the known range of WNS, and we found a wide diversity of *Geomyces* species, comprising around one third of all isolates. Many of these *Geomyces* species, along with numerous potentially novel lineages, appear to be undescribed.

Other Topics

689. Understanding the cellular basis of Azole resistance in Aspergillus fumigatus. Michael J. Bromley, Marcin Fraczek, Rebecca Collins, Emma Davies, Paul Bowyer. Translational Med, Univ Manchester, Manchester, United Kingdom.

Resistance of Aspergillus fumigatus to the azole class of antifungals is becoming a major problem in Europe and is being driven by two factors, the prolonged exposure (several months to several years) of patients to azoles and the extensive use of agricultural azoles driving environmental resistance. Our understanding of the mechanisms that govern azole resistance in filamentous fungi is limited. While some clinical resistant isolates harbor mutations

in the azole target, lanosterol 14 a-demethylase (cyp51A), more than 50% do not. We have used a combination of whole genome sequencing, transcriptomics, transposon based mutagenesis and high throughput directed mutagenesis to identify novel mechanisms that may explain the resistance observed in these strains. I will summarize and discuss our progress to date and present a worrying mechanism that results in both pan-azole and amphotericinB resistance.

690. Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*. D. A. Macdonald[1], A. E. Johns[1], M. Eberle[2], P. Bowyer[1], D. Denning[1], M. J. Bromley[1]. 1) Institute of Inflammation and Repair, Respiratory & Allergy Centre, University of Manchester, Manchester, United Kingdom; 2) Applied Microbiology, Institute for Applied Life Sciences, University of Karlsruhe, Hertzstrae 16, 76187 Karlsruhe, Germany.

Current drugs used to treat Aspergillus infections are limited and suffer from a variety of shortcomings including low efficacy, toxicity and increasing resistance. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the 'druggability' of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic to humans. Consequently most of the antimicrobials presently on the market were originally discovered by random screening of compounds against whole cell screens. A solution to this problem is to identify gene targets utilizing compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as a diploid strain lacking a single copy of a drug's target is hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80*$^{-}$*/Ku80*$^{-}$ mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated with several compounds. High-throughput methods of chemical genetic profiling by pooling multiple heterozygous KO strains into a single culture is currently being validated and preliminary data is promising. This will enable high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

691. Antifungal *Pisum sativum* defensin 1 Induces a non-Apoptotic Death in *Aspergillus nidulans*. Caroline M Fernandes[1], Luciano N Medeiros[1], Landi VG Costilla[1], Hilda Petrs-Silva[1], Patrícia A de Castro[2], Gustavo H Goldman[2], Eleonora Kurtenbach[1]. 1) Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Sao Paulo University, Ribeirao Preto, Sao Paulo, Brazil.

*Ps*d1 is a basic, cysteine-rich plant defensin isolated from *Pisum sativum* seeds which inhibits the growth of a broad range fungi species. Defensins are also non-toxic to mammalian cells, highlighting their potential as antifungal drugs. We have shown that FITC-labelled *Ps*d1 was internalized in *F. solani* hyphae, interacting with cyclin F and leading to fungal cell cycle arrest. This internalization seemed to be dependent of glucosylceramide (CMH, of cerebroside monohexoside), once *C. albicans* cells lacking the ceramide synthase are 25 % less susceptible to *Ps*d1 than the parental strain. Fungal and mammalian CMH are structurally divergent, as the former presents a C8-unsaturation and C9-methylation on the sphingoid base, which could possibly drive *Ps*d1 selectivity. In this work, we investigated the cell death mechanisms triggered by *Ps*d1 in *Aspergillus nidulans* and the contribution of CMH structure to *Ps*d1-induced fungal death. We characterized, through fluorescence microscopy, several apoptotic events, such as intense formation of reactive oxygen species (ROS), metacaspase activation and DNA strand breaks. Although *A. nidulans* hyphae treated with 20 mM Psd1 for 24 hours exhibited severe cell injury, no apoptosis-phenotype was observed. We also investigated whether *Ps*d1 incubation would lead to membrane permeabilization typical of a necrotic death. To this, *A. nidulans* cells were maintained in the presence or absence of the peptide and the membrane damage was evaluated through Propidium Iodide (PI) staining. We observed 15 % PI positive cells in the suspension treated with *Ps*d1, in contrast to 2 % in control culture. To investigate the role of fungal CMH and its structural modifications to *Ps*d1-induced cell death, we constructed strains lacking the glucosylceramide synthase (ANID_08806), sphingolipid DD8-desaturase (ANID_04592) and sphingolipid C9-methylase (ANID_05688 and ANID_07375) genes. Phenotype analysis showed impaired growth of strains deficient in ANID_08806 and ANID_04592 in comparison to the parental strain. Further investigation will be conducted to characterize *Ps*d1 antifungal activity and apoptosis or necrosis induction in the mutant strains. Unraveling the mechanisms of cell death induced by antifungal peptides may lead to the identification of new targets that drive antimycotic selectivity.

692. Is fungal secondary metabolism regulated by competing insects? Annika Regulin[1], Nancy Keller[2], Frank Kempken[1]. 1) Department of Botany, Christian-Albrechts University, Kiel, Germany; 2) Department Medical Microbiology and Immunology, Dept of Bacteriology, UW-Madison, USA.

Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxin) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4). In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. The vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with Drosophila larvae microarray analysis was performed. This led to the identification of secondary metabolite genes up- or down-regulated under these conditions. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression at different time points. Fungal single, double and triple mutations of identified up-regulated genes are currently analyzed in confrontation assays to identify potential modifications in gene expression and the survival rate of larvae concerning to chemical defense reaction of fungus-insect interaction compared to wild type. This could reveal insights about the biological function of secondary metabolite genes and clusters such as *stc* and *mdp*.
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693. Eisosome distribution and localization in the meiotic progeny of *Aspergillus nidulans*. A. Athanasopoulos[1], H. Boleti[2], C. Scazzocchio[3], V. Sophianopoulou[1]. 1) Institute of Biosciences and Applications, Microbial Molecular Genetics Laboratory, National Center for Scientific Research, Demokritos (NCSRD), Athens, Greece; 2) Intracellular Parasitism Group, Molecular Parasitology Laboratory, Department of Microbiology and Light

Microscopy unit, Institut Pasteur Hellenique, Athens, Greece; 3) Department of Microbiology, Imperial College, London, United Kingdom.

In the model filamentous fungus *Aspergillus nidulans*, PilA and PilB, two homologues of the *Saccharomyces cerevisiae* eisosome proteins Pil1/Lsp1, and SurG, a strict orthologue of Sur7, are assembled and form tightly packed structures in conidiospores. *A. nidulans* differs from the Saccharomycotina in that it has the ability to reproduce through two different types of spores, conidiospores and ascospores, cells which have a radically different morphology and are formed through completely different developmental pathways. Ascospores are formed only after the completion of meiosis inside asci, conidiospores arise from mitotic budding of specialized cells (phialides). We thus investigated eisosome composition and distribution in ascospores. Our results show that core eisosome proteins PilA, PilB and SurG are not expressed in hülle cells or early ascospores, but are expressed in mature ascospores. PilA forms static punctate structures at the plasma membrane as does PilB (with higher concentration at the areas where the two halves of ascospores are joined together), while SurG was localized both at the membrane and perinuclearly. In germlings originating from ascospores the punctate structures were shown to be composed only of PilA. In germinated ascospores PilA foci did not colocalise with the punctate structures of AbpA, a marker for sites of clathrin-mediated endocytosis. In the presence of myriocin -a specific inhibitor of sphingolipid biosynthesis- PilA-GFP foci of ascospore germlings were less numerous and their distribution was significantly altered. In this study we also investigated one of the two *A. nidulans* orthologues of Nce102, a protein that determines the structure and function of membrane microdomains in *S. cerevisiae*. In quiescent conidia localization of the closest orthologue, AnNce102 is detected in PilA plasma membrane associated foci as well as in 3-5 round-shaped intracellular structures. In early hyphae, a cytoplasmic fraction of Nce102 is additionally detected in highly dynamic structures that resemble Golgi equivalents. Deletion of core eisosomal genes causes mislocalization of Nce102 from the plasma membrane to these cytoplasmic structures. Ongoing experiments are investigating AnNce102 localization in response to sphingolipid biosynthesis and the nature of the intracellular compartments where it is located.

694. Fungal-bacterial interactions: *Bacillus subtilis* forms biofilm on *Aspergillus niger* hyphae. Isabelle Benoit[1,2], Marielle H. van den Esker[3], Miaomiao Zhou[1], Oscars P. Kuipers[3], Ronald P. de Vries[1,2], Ákos T. Kovács[4]. 1) Fungal Physiology, CBS-KNAW, Utrecht, Utrecht, Netherlands; 2) Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands; 3) Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; 4) Terrestrial Biofilms, Institute of Microbiology, Friedrich Schiller University, Jena, Germany.

Pure cultures of the filamentous fungi *Aspergillus niger* and *Aspergillus oryzae* and the Gram-positive bacterium *Bacillus subtilis* are widely used in industry for the production of pharmaceuticals, food ingredients and enzymes. However both by design and by accident, industrial fermentation can also involve mixed populations of micro-organisms. Moreover, in natural biotopes, these organisms live in mixed communities and have complex interactions ranging from competition to symbiosis. *B. subtilis*, in specific conditions, is capable of forming beneficial biofilms on surfaces and interfaces from plant roots to metal surfaces. In this study, co-cultivations of *A. niger* and *A. oryzae* together with *B. subtilis* were performed. *A. oryzae* inhibits *B. subtilis* growth while a bacterial coating was observed on *A. niger* hyphae. Microscopic and transcriptomic approaches were combined to study this fungal-bacterial interaction example.

695. Co-cultivations of fungi: microscopic analysis and influence on protein production. Isabelle Benoit[1,2], Arman Vinck[2], Jerre van Veluw[2], Thijs Gruntjes[1,2], Han A.B. Wösten[2], Ronald P. de Vries[1,2]. 1) Fungal Physiology, CBS-KNAW, Utrecht, Utrecht, Netherlands; 2) Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands.

During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *Aspergillus niger* and *Aspergillus oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. We have co-cultivated the wild type strains of these two Aspergilli with each other as well as the XlnR knock out strains. XlnR is a transcription factor inducible by the presence of xylose and responsible for the regulation of a variety of genes encoding plant polysaccharide degrading enzymes. The morphology and mechanism of the interaction of these cultures on wheat bran is addressed using microscopy and proteomics.

696. Improving heterologous protein production in *Aspergillus vadensis* . Ourdia Bouzid[1,2], Ronald P. de Vries[1,2]. 1) Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands; 2) CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.

Aspergillus vadensis is a good candidate for heterologous protein production, because it produces very low levels of extracellular proteases and does not acidify the medium. To improve protein production in *A. vadensis* two strategies were tested: (i) identification of new promoters for high gene expression, and (ii) overexpression of the xylanolytic regulator, XlnR. Six new *A. niger* constitutive promoters were selected and compared to the *gpdA* promoter using an arabinofuranosidase (*abf*) encoding gene from *Fusarium oxysporum* as a reporter. Several of the new promoters resulted in higher Abf activity than *gpdA*. For the second strategy, *A. vadensis* was transformed with *xlnR*, *xlnD* (encoding beta-xylosidase) and *faeA* (encoding feruloyl esterase) alone, and with combinations of *xlnD* and *xlnR*, and *faeA* and *xlnR*. Southern blot profiles confirmed the presence of multiple copies of the genes in the transformants. XlnD and FaeA activities were measured and were compared to the control strain. This demonstrated that increased copy numbers of *faeA* and *xlnD* had a much larger effect on the corresponding activities than increased copy numbers of *xlnR*. These data demonstrate that the new promoters in combination with high copy integration of the target genes can result in higher protein production by *A. vadensis*. Highlights from this study will be presented.

697. Production and characterization of esterases from *Chaetomium thermophilum* and their applicability in biomass conversion. Xiaoxue Tong, Peter Busk, Morten Grell, Lene Lange. Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering. Aalborg University Copenhagen, Denmark.

Xylan is the dominating hemicellulose constituent of plants and the most abundant renewable polysaccharide in nature after cellulose. Xylan and its hydrolysis products are potential resources for nutraceuticals, cosmetics, foods, bioalcohol, and industrial fine chemical production. Feruloyl esterase and acetyl xylan esterase are required for complete enzymatic hydrolysis of xylan due to its highly heterogeneous nature. The aim of this study was to produce and characterize esterases from the thermophilic fungus *Chaetomium thermophilum*. The esterase genes were identified by a novel bioinformatics tool PPR (Peptide pattern recognition, Busk & Lange, 2011). A Feruloyl esterase gene (*CtFaeA*) and a xylan esterase gene (*CtAxeA*) were successfully expressed in the yeast *Pichia pastoris*. They were purified to homogeneity from the culture supernatants. The effect of temperature and pH on the activity and stability of the esterases, as well as their substrate specificities, were studied. Both CtFaeA and CtAxeA displayed broad thermal stability and pH stability. Moreover, both esterases were active on hydrolysis of wheat arabinoxylan. These results show that *Chaetomium thermophilum* has a high capacity for degradation of xylan in addition to its already described cellulolytic potential. Furthermore, the robust esterases from *Chaetomium thermophilum* have

potential application in biomass bioconversion to e.g. higher value chemicals or biofuels.

698. Antioxidant adaptation by Eugenol and its derivatives and their affect on the expression of virulence in candida species. Aijaz Ahmad, Nikhat Manzoor. Bioscience, Jamia Millia Islamia, New Delhi, Delhi, India.

Present work investigates the antifungal activity and mode of action of eugenol (EUG), and its three derivatives- methyl eugenol, thymol and carvacrol. EUG and its derivatives were tested for antifungal activity by standard methods of CLSI. These varied in their mechanism of action depending upon the period of exposure. Short exposures of 5-15 minutes resulted in reduced H+ efflux by the H+-pump. From our studies we conclude that EUG and its derivatives induce production of free radicals which stimulates the enzyme SOD. An increased SOD activity resulted in an increase in the concentration of H2O2 which further stimulates the peroxide eliminating enzyme, primarily GPx. It is noteworthy that the levels of GSH an essential substrate of GPx were drastically reduced by the test compounds and this reduction gets even greater as increased levels of H2O2 decrease the activity of G6PDH which provides reducing equivalents to GR, an enzyme that recycles GSH from GSSG. Decreased G6PDH activity aids further in the reduction of GSH. Again, reduced availability of GSH explains decreased GPx activity. Another enzyme to eliminate H2O2 is catalase, which triggers a cellular response leading to an increase in its activity. Hence increase in the activity of two important antioxidant enzymes SOD and catalase, clearly demonstrates an increase in the concentration of ROS when the Candida were exposed to the EUG and derivatives. These enzymatic responses were not enough to defend the cell completely against a high rise in ROS and therefore did not meet the required cellular antioxidant demand. Ultimately, the outburst of free radical production led to severe lipid peroxidation. Cell death on exposure to EUG and its derivatives hence may be due to (i) decrease in the rate of H+efflux (ii) reduced ergosterol content (iii) Induction of oxidative stress in the cell (iv) These processes impair membrane structure and function which form lesions. Infection process of Candida is characterized by crucial pathogenicity markers. The process of germ tube induction followed by the secretion of hydrolytic enzymes help in the invasion of the host cells. The expression profile of selected genes associated with Candida virulence by RT-PCR showed a reduced expression of HWP1, SAP1 and PLB2 genes in Candida treated with EUG and its derivatives.

699. Elevation of chitin is linked with multiparallel mechanisms in response to *C. albicans* cell wall stress. F. Nogueira, L. Walker, C. Munro, N. Gow. Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

The role of the MAPK, Ca^{2+}/calcineurin and cAMP/PKA signal transduction pathways in regulating the *Candida albicans* cell wall stress response was investigated. A library of mutants lacking receptors, signalling elements and transcription factors were screened for alterations in the ability to respond to a range of cell wall stressing agents, including $CaCl_2$, Calcofluor White and caspofungin. Pre-treatment of wild-type cells with $CaCl_2$ and CFW, activates the Ca^{2+}/calcineurin and PKC pathways, leading to an increase in chitin content, and reduced susceptibility to caspofungin. Although elevation of cell wall chitin content often resulted in decreased sensitivity to caspofungin, we show here that some strains with increased chitin levels remained sensitive to caspofungin. The results show that elevation of chitin is a common property of a range of mutants that are affected in coordinating cell wall stress pathways, but that multiple mechanisms are likely to operate in maintaining the robustness of the *C. albicans* cell wall.

700. Prezygotic and postzygotic control of uniparental mitochondrial DNA inheritance in *Cryptococcus neoformans*. Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Uniparental inheritance of mitochondrial DNA is pervasive in non-isogamic higher eukaryotes during sexual reproduction and postzygotic and/or prezygotic factors are shown to be important in ensuring such inheritance pattern. Although the fungus *Cryptococcus neoformans* undergoes sexual production with isogamic partners of opposite mating types **a** and a, most progeny derived from such mating events inherit the mitochondrial DNA from the **a** parent. The homeodomain protein complex Sxi1a/Sxi2**a**, formed in the zygote after **a**-a cell fusion, was previously shown to play a role in this uniparental mtDNA inheritance. Here, we defined the timing of the establishment of the mtDNA inheritance pattern during the mating process and demonstrated a critical role in determining the mtDNA inheritance pattern by a prezygotic factor Mat2. Mat2 is the key transcription factor that governs the pheromone sensing and response pathway, and it is critical for the early mating events that lead to cell fusion and zygote formation. We show that Mat2 governs mtDNA inheritance independent of the postzygotic factors Sxi1a/Sxi2**a**, and the cooperation between these prezygotic and postzygotic factors help achieve stricter uniparental mitochondrial inheritance in this eukaryotic microbe.

701. SIS, a sex genome defense mechanism operating in *Cryptococcus neoformans*. Xuying Wang, Sabrina Darwiche, Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans is a human fungal pathogen that undergoes a dimorphic transition from yeast to hyphae during **a**-a opposite-sex mating and a-a unisexual reproduction (same-sex mating). Infectious spores are generated during both processes. We previously identified a sex induced silencing (SIS) pathway in the *C. neoformans* serotype A var. *grubii* lineage, in which tandem transgene arrays trigger RNAi-dependent gene silencing at a high frequency during **a**-a opposite-sex mating, but at an ~250-fold lower frequency during asexual mitotic vegetative growth. Here we report that SIS also operates during a-a unisexual reproduction. A self-fertile strain containing either *SXI2***a**-*URA5* or *NEO-URA5* transgene arrays exhibited an elevated silencing frequency during solo and unisexual mating compared with mitotic vegetative growth. We also found that SIS operates at a similar efficiency on transgene arrays of the same copy number during either a-a unisexual reproduction or **a**-a opposite-sex mating. *URA5*-derived small RNAs were detected in the silenced progeny of a-a unisexual reproduction and RNAi core components were required, providing evidence that SIS induced by same-sex mating is also mediated by RNAi via sequence-specific small RNAs. This study, together with our previous finding of SIS in **a**-a opposite-sex mating of the *C. neoformans* serotype A var. *grubii* lineage, demonstrates that SIS is a conserved process between the divergent *C. neoformans* serotype A and serotype D sibling species. In each case, our data show that the SIS RNAi pathway operates to defend the genome via squelching transposon activity during the sexual cycles. Thus, our discovery of SIS brings a fresh perspective to meiotic silencing involving the upregulation of RNAi pathways as a strategy to guard genomic integrity during sex. More importantly, the presence of SIS in both a-a unisexual reproduction and **a**-a opposite-sex mating indicate that SIS may be triggered by the shared pheromone sensing Cpk1 MAPK signal transduction cascade. Ongoing studies focus on defining at a mechanistic level how the SIS RNAi pathway is initiated, including identifying new components involved in SIS.

702. Effects of the use of biocontrol agent (*Phlebiopsis gigantea*) on fungal communities of *Picea abies* stumps. E. Terhonen[1], H. Sun[1], M. Buée[2], R. Kasanen[1], L. Paulin[3], F. Asiegbu[1]. 1) University of Helsinki, Department of Forest Sciences, P.O.Box 27, FIN-00014, University of Helsinki, Finland; 2) INRA, UMR 1136 INRA/Nancy Université Interactions Arbres/Microorganismes, INRA-Nancy, 54280 Champenoux, France; 3) DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland.

The saprotrophic fungus *Phlebiopsis gigantea* has for several years been used as a biocontrol agent against pathogen *Heterobasidion annosum*. This pathogen is the major cause of root rot disease in conifers that results in economic losses estimated at 50 million euros to Finnish forestry. A major

problem is that, although the effectiveness of *P. gigantea* as a bio-control agent has empirically been shown, the long term biological effect of this fungus on conifer trees as well as on other soil micro-flora has not been empirically proven. We investigated the impact of *P. gigantea* treatment on stump mycobiota using metagenomic pyrosequencing approach as this has not been done before. Samples from forest sites pre-treated with *P. gigantea* for 1, 6 and 13 years ago were collected, DNA was isolated and pyrosequenced. Similarly samples were also collected from untreated stumps within the same forest sites. Sequences were quality trimmed using Mothur software. After trimming we had 26 398 sequences from 53 117. For the extraction of the full ITS1 of the nuclear ITS region FungalITSextractor was used and these sequences were clustered at 97% similarity using cdhit-454 with the most abundant sequence types serving as cluster seeds. The most frequent sequence type in each cluster was used for the BLAST searches against NCBI BLASTN and ³ 97% similarity across the entire length of the pairwise alignment was taken to indicate conspecificity. Differences between control and treated stumps were tested statistically with Paired-Sample T-test (SPSS 19). Also diversity indexes and similarity indexes between controls and treated were calculated using Estimates 8.2.0. After one year of the clear-cut we found from *Phlebiopsis gigantea*-treated stumps 107 different fungal OTUs and from non-treated stumps 119 fungal OTUs, from which they shared 102 OTUs. After 6 years we observed from treated stumps 118 fungal OTUs and from non-treated 134 fungal OTUs and they shared 99 OTUs. After 13 years we found from treated stumps 131 OTUs and from non-treated 139 OTUs and shared OTU number were 109. However there were no statistical differences between control and treatment. Based on our results our primary conclusion is that stump treatment should continue as there is no obvious adverse effect on the other stump mycobiota.

703. Diversity of yeast and mold species in different cheeses. Nabaraj Banjara[1], Kenneth Nickerson[2], Heather Hallen-Adams[1]. 1) Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE; 2) Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

The yeast and mold diversity from different commercial cheeses collected from local markets (Lincoln, NE, USA) was studied using microbial counting and molecular biology approaches. Twenty one distinct types of cheese samples were investigated. Briefly, 10 grams of each cheese sample was homogenized in distilled water, serially diluted from 10 to 10^{-6}, grown in Yeast Extract Glucose Chloramphenicol Agar (YGC) and the population was counted from dilution plates. Yeasts and molds were identified by amplification and sequence analysis of the nuclear ribosomal RNA genes, using ITSIF and TW13 primers. *Debaryomyces hansenii* was the predominant fungal species in most of the cheeses (59% of samples at up to 5.7 x 10^6 CFU/ gram). Other fungi isolated included *D. fabryi, D. prosopidis, D. subglobosus, Penicillium roqueforti* and *Candida sake*. In our samples, farmstead cheese had the highest (2.1 x 10^8 CFU/gram) and Swiss cheese the lowest (8x10^3 CFU/gram) fungal population.

704. Heterologous expression and characterization of soil organic matter-specific proteases secreted by the ectomycorrhizal fungus *Paxillus involutus*. Morten N. Grell[1], Linas Pupelis[1], Tomas Johansson[2], Firoz Shah[2], Lene Lange[1], Anders Tunlid[2]. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Microbial Ecology Group, Department of Biology, Lund University, Sweden.

Paxillus involutus (Batsch) Fr. (Basidiomycetes; Boletales) is widely distributed in the Northern hemisphere, and is one of the best-studied ectomycorrhizae fungi, especially with respect to its ecology and physiology. In a study on the mechanisms by which *Paxillus involutus* degrade complex organic matter extracted from plant litter material, transcriptomes were sequenced using the 454 technology and NimbleGen microarrays produced. A number of genes encoding extracellular enzymes showed an increased transcript level during degradation of soil organic matter (SOM), as compared with growth on a defined medium (MNM). They were suggested to constitute a Fenton-like, radical-based biodegradation system that disrupts the organic matter-protein complexes thereby mobilizing embedded nitrogen (Rineau et al. 2012, Environm. Microbiol. 14, 1477-1487). Supporting this, a number of protease genes were found to have a significantly increased SOM/MNM expression value and to be upregulated during growth on protein-rich substrates. Most highly expressed were aspartic, metallo, and serine proteases. This is in agreement with biochemical analysis of SOM degradation. To study substrate specificity and regulation of selected proteases in detail these are expressed in the yeast *Pichia pastoris*.

705. Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance. Therese Wohlschlager[1], Stefanie Schmieder[1], Alex Butschi[2], Paola Grassi[3], Alexander Titz[4], Stuart Haslam[3], Michael Hengartner[2], Markus Aebi[1], Markus Künzler[1]. 1) Institute of Microbiology, ETH Zürich, Switzerland; 2) Institute of Molecular Life Sciences, University of Zürich, Switzerland; 3) Division of Molecular Biosciences, Imperial College, London, United Kingdom; 4) Department of Chemistry, University of Konstanz, Germany.

The defense of fungi against fungivores is largely based on the production of intracellular toxins. A significant proportion of these toxins are peptides and proteins that are synthesized by the ribosome and stored in the cytoplasm. Protein toxins include lectins that target specific glycoepitopes in the intestine of the fungivore upon ingestion and kill the fungivore by a yet unknown mechanism. In our laboratory, we focus on the functional characterization of fungal protein toxins that are directed against nematodes. We use the model nematode *Caenorhabditis elegans* to identify the targets and to study the toxicity mechanism of these fungal defense effector proteins in the nematode. In addition, we employ the fungivorous nematodes *Aphelenchus avenae* and *Bursaphelenchus willibaldi* to study the diversity, the functional significance and the transcriptional regulation of these proteins in the fungus. Recently, we identified a nematotoxic lectin from the mushroom *Laccaria bicolor* that is homologous to animal lectins involved in innate immunity against bacteria. We found that the nematotoxicity of the lectin is based on its specific binding to methylated fucose residues on nematode N-glycans. Among animals, this epitope is only present in worms and molluscs but not in insects or vertebrates. We performed affinity chromatography of *C. elegans* whole worm protein extracts using the *L. bicolor* lectin and other nematotoxic fungal lectins recognizing protein-bound glycans. The results of this analysis suggest that these lectins target the same set of glycoproteins in the nematode intestine and may confer toxicity by a common mechanism. In order to address the functional significance of these proteins for fungal defense against fungivorous nematodes, we expressed some of the fungal proteins displaying toxicity towards *C. elegans*, in the filamentous ascomycete *Ashbya gossypii*. These transformants were fed to *A. avenae* and the propagation of the fungivorous nematode on the various transformants was determined. Expression of some effector proteins significantly inhibited propagation of the nematode suggesting that these proteins have a role in fungal defense against these organisms. Experiments addressing the relative fitness of the various *A. gossypii* transformants upon selective pressure of feeding by *A. avenae* are under way.

706. Microfluidic platforms for monitoring interactions between fungi and bacteria. Martina A. Stöckli[1], Claire E. Stanley[2], Pauli T. Kallio[1], Markus Künzler[1], Andrew J. deMello[2], Markus Aebi[1]. 1) Microbiology and Immunology, ETH Zurich, Zurich, Switzerland; 2) Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland.

Bacteria and fungi share many microhabitats where they interact with each other. These interactions are diverse and play important roles in certain human infections, as well as in the biological control of plant diseases. The basis of these interactions, however, is not well understood, as their investigation is technically challenging. Conventional microscopic approaches are limited, in that they can only monitor interactions at a particular time

point and are not able to follow dynamic interactions. Herein, the development of a microfluidic platform, to study the interplay of individual hyphae with bacteria in a confined compartment, is detailed. A microfluidic device, comprised of single, interconnected microchannels, is filled with medium. The device is manufactured from the polymer, polydimethylsiloxane, which is bonded to a glass layer. Importantly, this polymer possesses some desirable characteristics, making it compatible with experiments of biological nature, for example it is oxygen permeable, and allows optical detection from 240 nm to 1100 nm. The fungus can easily grow into the microchannels from an agar plug that is placed next to a lateral opening. Subsequently, the device can be co-inoculated with bacteria through a separate inlet, allwoing changes in morphology, growth rate, and interaction patterns of the same hyphae upon the addition of bacteria to be investigated over time. As an example, the interaction of the basidiomycetous model organism, Coprinopsis cinerea, and the gram-positive bacterium, Bacillus subtilis, which are both found in dung of herbivores, has been studied. Using these microfluidic platforms, it has been observed that B. subtilis cells attach to hyphae in an end-on manner. The frequency of attachment within hyphae and between different hyphae varies. Furthermore, the tips of the hyphae are not colonised by the bacterial cells. In summary, this approach can be used to study phenotypic changes of bacteria and fungi over specific time periods. In future, it is envisaged that this data will be combined with fungal and bacterial genetic approaches.

707. WITHDRAWN

708. The French *Fusarium* Collection: a living resource for mycotoxin research. L. Pinson-Gadais, M. Foulongne-Oriol, N. Ponts, C. Barreau, F. Richard-Forget. INRA, UR1264-MycSA, 71 avenue Edouard Bourlaux, F-33883 Villenave d'Ornon, France.

Fusaria are responsible for prejudicial diseases on cereal crops worldwide, such as crown rot and *Fusarium* head blight. Beyond economic losses due to infection symptoms, these pathogens can produce several types of mycotoxins that are harmful to livestock and humans. They are extremely diverse at the intra-specific levels in terms of types as well as quantities of toxins that a strain can produce. Developing appropriate strategies to limit contamination with *Fusarium* mycotoxins requires a greater knowledge about this variability. We have collected a large number of toxinogenic *Fusarium* strains. Our assortment now includes about 800 strains, mostly from the species *graminearum*, *culmorum*, *verticilloides*, *proliferatum*, and *temperatum*. Species were identified based on morphology and real-time PCR. More than half of our strains were further characterized for toxin production using biochemical and/or real-time PCR-based tools. We isolated about 70 *F. graminearum* strains from either wheat or maize grains originating from different French cereal production areas. Our results show a high representation of 15-acetyldeoxynivalenol-producing strains in our French samples. Within the same chemotype, we observe a large variability in toxin production levels. The *F. graminearum* strains were characterized with microsatellite markers and show a large genetic diversity. Two groups were delineated according to their genetic background, roughly corresponding to strains isolated from Europe in one hand and America in the other hand. Our results are also in agreement with the fact that only *F. graminearum* sensu stricto strains seem to be detected in France so far. The demonstrated genetic and phenotypic diversity provides a sound ground for countless downstream studies such as genetic association and quantitative genetics to understand the determinism of toxin production. Such information should be doubtlessly considered in plant breeding efforts and other disease management strategies aimed at reducing the mycotoxin risk in food and feeds. Our collection is a valuable tool to improve our understanding of toxigenic diversity in *Fusarium* species. It is managed through a database gathering all information collected on each strain, already available upon request and soon publically available as a web-based interface.

709. Chemical genetics: Discovery of novel fungicides and their targets in the phytopathogen Fusarium graminearum. G. Subramaniam, C. Mogg. Agriculture Canada, Ottawa, ON, Canada.

Chemical genetics screen is based on the ability of small chemical molecules to bind to biological molecules and alter their function. Screening of pharmaceutical libraries has revealed novel molecules effective against cancer and other diseases. We have adopted similar approach and identify bio-active compounds that will block the growth and development of *F. graminearum*. We have developed a 96-well format to monitor the growth of *F. graminearum* in liquid media. The fungus is tagged with a green fluorescent protein (GFP) and the growth is monitored by the measurement of fluorescence of the GFP. This format facilitates high throughput screening for small molecules that could potentially disrupt the growth of the fungus. As proof of concept, we screened ~560 compounds from the TimTec NDL-3000 natural product collection (TimTec LLC, Newark, DE, USA) and identified several compounds with anti-Fusarium properties. One compound identified form our screen, "Antofine" was purified from *Vincetoxicum rossicum* and was used in subsequent studies, to identify targets in the fungus. We used the gene deletion library of the budding yeast *Sacchromyces cerevisiae* to identify targets for Antofine. GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions of these targets. Our efforts to identify targets in Fusarium against Antofine will be discussed.

710. Functional characterization of an *Aspergillus flavus* polyketide synthase gene necessary for the synthesis of a sclerotium-specific pigment. J.W. Cary[1], P. Harris-Coward[1], K.C. Ehrlich[1], P. Dowd[2], S. Shantappa[3], A.M. Calvo[3]. 1) US Department of Agriculture, ARS-SRRC, New Orleans, LA; 2) US Department of Agriculture, ARS-NCAUR, Peoria, IL; 3) Northern Illinois University, DeKalb, IL.

The filamentous fungus, *Aspergillus flavus*, produces the toxic and carcinogenic, polyketide-derived family of secondary metabolites termed aflatoxins (AFs). In addition to the AF biosynthetic gene cluster, analysis of the *A. flavus* genome has identified 55 gene clusters predicted to be associated with secondary metabolism. To date, very few of the metabolites produced by these clusters have been identified. Secondary metabolism is controlled by global regulators such as LaeA and VeA. In a *veA* knockout mutant we identified a significantly down-regulated polyketide synthase (PKS) gene belonging to cluster 27. Although the metabolite produced by this cluster was unknown, *in silico* cluster analysis predicted that cluster 27 would consist of the PKS gene and four other genes. qRT-PCR analysis confirmed that expression of the cluster 27 PKS (*pks27*) gene was down-regulated in the *veA* mutant. Inactivation of the *pks27* gene resulted in loss of the dark pigment associated with *A. flavus* sclerotia. Sclerotia are survival structures produced by condensation of mycelia and function as propagules in the field. Conidial pigmentation did not appear to be affected in the *pks27* knockout strain. TLC and HPLC analysis of sclerotial extracts identified the cluster 27 metabolite as asparasone A. Insect feeding studies using wild-type and mutant sclerotia indicated that the pigment may be acting as a feeding deterrent. To our knowledge this is the first report on the identification of a gene that encodes a sclerotium-specific pigment. The pigment likely plays a role in sclerotial resistance to insect feeding and possibly other environmental stresses.

711. Functional Analysis of the *Pleurotus ostreatus* Manganese-Peroxidase Gene Family. Tomer Salame, Doriv Knop, Dana Levinson, Oded Yarden, Yitzhak Hadar. Microbiology and Plat Pathology, Hebrew Unversity, Rehovot, Israel.

Mn amendment to *P. ostreatus* cultures enhances degradation of recalcitrant aromatic compounds. Manganese peroxidase (MnP) isoenzymes are key players in these processes. The MnP gene family is comprised of five Mn -dependent peroxidases (mnp3, 6, 7, 8 and 9) and four versatile-peroxidases (mnp1, 2, 4 and 5; VPs). In liquid medium, Mn amendment resulted in a drastic up-regulation of the predominantly expressed mnp3 and mnp9, and down-regulation of mnp4. To obtain direct evidence for the role of these enzymes, we produced genetically-modified (knockout, knockdown and/or over-

expression) strains in *mnps* and studied their degradation capacity. The compounds studied were: azo-dyes such as orange II and reactive black, recalcitrant pharmaceutical compounds found in treated waste water such as Carbamazepine and lignocellulosic agricultural waste. We engineered a transformant, constitutively expressing mnp4 a VP naturally repressed by Mn (designated OEmnp4) under the control of the b-tubulin promoter. Now, despite the presence of Mn in the medium, OEmnp4 produced mnp4 transcript as well as VP activity as soon as four days after inoculation. OEmnp4 decolorized the azo-dyes two days earlier relative to the wild type in Mn amended medium. RNAi silencing targeting mnp3 resulted in a delay in the decolorization capacity which occurred concomitantly along with a marked reduction of the expression level of all mnps, particularly mnp3 and mnp9. This observation supported the conclusion that MnPs are involved in the process but could not determine the specific contribution of the different genes to the outcome. Therefore we produced a Dku80 strain, exhibiting a 100% homologous DNA recombination rate, to enable specific gene replacement. Subsequently, homokaryon mnp2, 3, 4 and 9 knockout strains were produced. In Mn amended GP, orange II decolorization was not significantly inhibited by any of these strains, indicating on functional redundancy. In Mn deficient GP, inactivation of mnp4 proved that it encodes the key VP responsible for Mn dependent and Mn independent peroxidase activity, as well as resulted in reduction of the azo dye reactive black 5 decolorization capacity. The tools and protocols developed increase the amenability of *P. ostreatus* to genetic manipulations and expand options for gene function analyses.

712. Temperature- and pH characteristics of endo-cellulases in Rhizophlyctis rosea. Bo Pilgaard[1], Frank Gleason[2], Peter Busk[1], Lene Lange[1]. 1) Section for sustainable biotechnology, Aalborg University- Copenhagen, Denmark; 2) School of Biological Sciences, University of Sydney, New South Wales, Australia.

The zoosporic true fungi of the order Chytridiales are of special interest since they constitute a central root position of the entire fungal kingdom. Enzymes of the anaerobic zoosporic rumen fungi have been studied quite extensively, but only scarce information is available about enzymes from aerobic zoosporic true fungi from soil. We have previously confirmed the presence of cellulases in Rhizophlyctis rosea (AUS13), which was isolated from soils of the Sydney Basin. Other studies have shown that this fungus can survive and grow within a wide pH-range (Gleason et. al 2010) and high temperatures (Gleason et. al 2005). We investigate the cellulolytic potential of Rhizophlyctis rosea in several cellulase assays and characterize the enzymatic properties with respect to temperature and pH stability of the enzymes. Moreover, we are sequencing the Rhizophlyctis rosea genome in order to find the genes for cellulose-degrading enzymes (GH6, GH7, GH45, GH6) that are present in Chytrids as compared to the cellulases of these families found in other fungal groups.

713. Applying unconventional secretion of the endochitinase Cts1 to export heterologous proteins in *Ustilago maydis* . J. Stock, P. Sarkari, M. Knopp, S. Jankowski, S. Bergmann, M. Feldbrügge, K. Schipper. Institute for Microbiology, Heinrich-Heine University Düsseldorf, 40204 Düsseldorf, Germany.

The demand on the biotechnological production of proteins for pharmaceutical, medical and industrial applications is steadily growing. Not every protein can easily be produced by the existing platforms. For the production of such challenging proteins, we aim to establish a novel expression system in the well characterized eukaryotic microorganism *Ustilago maydis*. In this fungus, secretion of the endochitinase Cts1 depends on mRNA transport along microtubules, which is mediated by the key RNA-binding protein Rrm4. We recently demonstrated that Cts1 secretion occurs via a novel unconventional route. We used b-glucuronidase (Gus) as a reporter for unconventional secretion. This bacterial enzyme is inactivated by N-glycosylation during its passage through the conventional eukaryotic secretory pathway. By contrast, in our system Gus was exported in its active form by fusion to Cts1 confirming its secretion by an unconventional route. Furthermore, we showed that this secretory mechanism can be exploited for the export of active heterologous proteins. As a proof-of-principle for economically important biopharmaceuticals we expressed an active single-chain antibody. Importantly, the novel protein export pathway circumvents N-glycosylation which is advantageous in many applications, for example to avoid undesired immune reactions in humans. Currently, the system is optimized with respect to product yield by e.g., reducing the proteolytic activity in the culture supernatants. Thus, the unconventional Cts1 secretion machinery has a high potential for the production of biotechnologically relevant proteins.

714. A new method for fungal genetics: flow cytometry of microencapsulated filamentous microcolonies. L. Delgado-Ramos[1], A. T. Marcos[1], X. Peñate[1], M. S. Ramos-Guelfo[1], L. Sánchez-Barrionuevo[1], F. Smet[2], D. Canovas[1], S. Chávez[1]. 1) Dept of Genetics, Univ of Sevilla, Spain; 2) Union Biometrica, Geel, Belgium.

Genetic analysis of non-filamentous microorganisms is facilitated by the isolation of consistent, well-defined colonies on solid media and the handling of individual cells by flow cytometry. In contrast, some filamentous fungi are hard to be analyzed using these procedures; in particular by flow cytometry. The combination of single spores microencapsulation and large particle flow cytometry is a possible alternative for the analysis of filamentous fungi. Microencapsulation allows the early detection of fungal growth by monitoring the development of hyphae from encapsulated individual spores. Mycelium proliferation inside the microcapsules can be detected using COPAS™ large particle flow cytometry. Here we show the successful application of the Flow Focusing® technology to the microencapsulation of filamentous fungi in monodisperse alginate microspheres, using *Aspergillus* and *Trychoderma* as model systems. Using a Cellena® Flow Focusing microencapsulator, we managed to produce monodisperse microparticles containing individual spores and to develop microcolonies of these fungi upon germination in the appropriate conditions. Proliferation inside the particles was monitored by microscopy and large particle flow cytometry without requiring fluorescent labeling. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations. Conditional mutants were utilized to demonstrate the feasibility of the method. This procedure allows for the handling, screening and analysis of clonal colonies in liquid culture. Examples of applications will be provided.

715. DNA methylation dynamics during development in the rice blast fungus. Junhyun Jeon[1], Jaeyoung Choi[2], Gir-Won Lee[2], Sook-Young Park[3], Aram Huh[1], Ralph Dean[4], Yong-Hwan Lee[1,2,3,5]. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea; 2) Fungal Bioinformatics Laboratory, Seoul National University, Seoul 151-921, Korea; 3) Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; 4) Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, USA; 5) Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921, Korea.

Cytosine methylation is an important epigenetic modification of DNA that is involved in genome defense and transcriptional regulation in eukaryotes. In mammals and plants, many roles of DNA methylation depend on dynamic changes of DNA methylation pattern. In fungi, DNA methylation is considered primarily as a stable mark for silencing transposable elements. Here we used genetic manipulations and high-throughput bisulphite sequencing on the model plant pathogenic fungus, *Magnaporthe oryzae* to elucidate the dynamics and mechanics of DNA methylation during pathogenic development. We found that genome-wide reprogramming of DNA methylation in and around genes occurs during progression of fungal development and that such reprogramming is important for normal development. RNA-seq analysis showed that DNA methylation is associated with transcript abundance of genes in context-dependent manner. Our study reveals that DNA methylation in fungi could be a dynamic epigenetic entity that has assumed new roles in developmental processes other than genome defense.

716. pH-enotype array: a novel phenomics platform for filamentous fungi. Jaejin Park[1], Yong-Hwan Lee[1,2]. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea; 2) Center for Fungal Pathogenesis, Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Rapid increase of genome information and application of high-throughput mutagenesis technology allowed large-scale gene characterization in filamentous fungi. However, phenotype screening is a bottleneck as it entails time-consuming and labor-intensive processes. Thus there is a demand for a novel platform for high-throughput phenotype screening. Although several microplate-based assays are currently available, their application on organisms growing in filamentous form has been met with considerable difficulty due to uneven distribution of cells. As a solution, we developed pH-enotype array - a new phenomics platform in filamentous fungi. This platform is based on the pH change in the culturing media, reflecting viability and physiological status of cells. The pH in culturing media is continuously measured by a microplate spectrophotometer using two pH indicators, bromocresol purple and phenol red. The validity of the system was comprehensively evaluated with *Magnaporthe oryzae* strains and we confirmed that the growth responses to various stresses or nutrient conditions can be characterized reliably within 24 hours using the optimized medium. This phenomics platform would provide a novel, high-throughput phenotype screening method in filamentous fungi and promote phenotype standardization for comparative functional genomic studies.

717. GFP analysis of meiotic recombination in Neurospora D*msh-2* homozygotes. P Jane Yeadon, Frederick J Bowring, David E A Catcheside. Sch Biological Sci, Flinders Univ, Adelaide, South Australia, Australia.

We have confirmed that, as expected for a mismatch repair gene with both vegetative and meiotic roles, the phenotype of a *msh-2* deletion in *Neurospora crassa* is recessive. Chromatid data indicates that deletion of *msh-2* increases allelic recombination at *his-3* by a factor of 1.6 with no effect on crossing over in the *lys-4* to *ad-3* interval in which lies both *his-3* and the recombination hotspot *cog*. Although analysis of a small number of octads from an *msh-2* deletion cross suggested that the only non-Mendelian segregation is post-meiotic, the ease by which octads can be scanned for recombination events under a fluorescent microscope when GFP is inserted close to *cog* allowed us to reveal the wide range of recombination outcomes normally hidden by the activity of Msh-2. We report that a degree of mismatch repair is retained in the absence of Msh-2 and that recombination initiated by *cog* exhibits a strong bias for repair in the direction of restoration rather than conversion. In contrast to recombination events in budding yeast, symmetric heteroduplex appears to be frequent in the *his-3* region, suggesting variation between recombination pathways utilised in the two species.

718. Residual recombination in *Neurospora crassa spo11* mutant homozygotes occurs during meiosis. Frederick J Bowring, P Jane Yeadon, David E A Catcheside. Sch Biological Sci, Flinders Univ, Adelaide, South Australia, Australia.

We have previously shown that although most genomic regions of Neurospora *spo11^{RIP}* mutants lack meiotic recombination, crossing over in the *his-3* region persists at close to wild-type levels. However, this residual recombination could conceivably occur after meiosis, during a transient period of partial diploidy. Using crosses heteroallelic for *his-3* mutations, we have shown that in *spo11^{RIP}* homozygotes, as in wild type, stable His$^+$ progeny are generated at high frequency. We have utilised mutations in either end of a histone H1-GFP fusion gene, inserted between the recombination hotspot *cog* and *his-3*, to show that the frequency at which GFP$^+$ spores arise in a cross homozygous for *spo11$^+$* is comparable to the frequency of His$^+$ spores, and that glowing nuclei first appear during pachytene, as expected for a product of meiotic recombination. In similar *spo11* deletion homozygotes, GFP$^+$ spores also arise at high frequency and glowing nuclei are also first seen at pachytene. Thus, *spo11* mutant homozygotes experience both crossing over and allelic recombination during meiosis, suggesting there is a *spo11*-independent mechanism for initiation of recombination at *his-3*.

719. Controlled synthesis of gold nanoparticles by *Neurospora crassa* extract and their SERS properties. Katrin Quester[1], Ernestina Castro-Longoria[1], Miguel Avalos-Borja[2,3], Alfredo Rafael Vilchis-Nestor[4], Marco Antonio Camacho-López[5]. 1) Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, B.C. México; 2) Centro de Nanociencias y Nanotecnologia, UNAM. Ensenada, B.C. México; 3) División de Materiales Avanzados, IPICyT. San Luis Potosí, S.L.P. México; 4) Centro Conjunto de Investigación en Química Sustentable, UAEMex. Toluca, Estado de México, México; 5) Laboratorio de Investigación y Desarrollo de Materiales Avanzados, Sección de Espectroscopía Raman, Facultad de Química-UAEMex, Unidad Rosedal. Toluca, Estado de México, México.

Nanotechnology, the study of the controlling matter of an atomic and molecular scale, has emerged as an interesting and important scientific field and the controlled synthesis of nanostructures from different chemical composition as well as their shape, size, and dispersity are important areas of research. The so-called 'green chemistry' or 'nanobiotechnology' employs microorganisms to fabricate nanostructures and has the benefit of improving the biocompatibility of nanomaterial, however the control over average particle size and uniform particle morphology is required but still a challenge. This work bases on the use of *Neurospora crassa*, a non-pathogenic filamentous fungus with rapid growth rate, for the biosynthesis of gold nanoparticles of controlled size and shape. Briefly, the fungal extract was incubated with the gold precursor solution at different conditions of temperature, pH and time of reaction. The best results obtained were from incubations at 60°C; at pH 3, particles of different shapes (e.g. spheres, triangles, hexagons, pentagons, rhombs and bars) were formed while at pH 5.5 and pH 10 small quasi-spherical particles were formed with size ranges of 6 to 21 nm and 3 to 12 nm, respectively. High resolution transmission electron microscopy (HRTEM) using a FEI Tecnai F30 transmission electron microscope confirmed the crystalline and elemental character of the gold nanoparticles. The synthesized gold nanoparticles of different shapes were shown to possess excellent surface-enhanced Raman scattering (SERS) enhancement ability relative to quasi-spherical gold nanoparticles. Small quasi-spherical nanoparticles of 3 to 12 nm enhances the Raman signals of methylene blue about 2 times, those of 6 to 23 nm enhance the Raman signals about 25 times whereas nanoparticles of different shapes with a broad size range enhances the Raman signals of methylene blue about 40 times. Results are promising and show that these gold nanoparticles might have potential applications for biological sensing and labeling systems.

720. Cellulase production in *Neurospora crassa*. M. Reilly, L. Glass. Energy Biosciences Institute, University of California, Berkeley, CA.

The filamentous fungus *Neurospora crassa* is a well-studied model organism that is frequently isolated from the environment in association with burned vegetation. Enzyme activities related to the metabolism of plant cell wall material have been observed in *N. crassa*, but the overall cellular response of the organism to such a recalcitrant carbon source remains poorly defined. In order to investigate the elements involved in fungal growth on cellulose, we utilized the near full genome collection of single gene knockout strains that has been generated under the auspices of the Neurospora Genome Project. Strains with deletions in loci thought likely to play a role in cellulase activity or production - including the known secretome of *N. crassa* when cultured on plant biomass, homologs of the *Saccharomyces cerevisiae* secretion apparatus, and proteins predicted to traverse the secretory pathway - were singled out for analysis. This subset of the *N. crassa* deletion collection was cultured on a cellulosic substrate and the levels of secreted protein and cellulase activity were compared to wild-type. A number of hyper- and hypo-secretion mutants have been identified. Sequence analysis suggests that while many of the loci encode fungal-specific proteins of undetermined functions, some of the deleted genes likely act in transcription, protein synthesis, and intracellular trafficking. Early work with the latter found that their hyper- or hypo-secretion phenotypes were a specific response to cellulose, but not

limited to the production of cellulolytic enzymes. The continuing characterization of these mutants will enhance our understanding of the ability of *N. crassa* to utilize the complex carbon sources present in its natural environment.

721. Identification and Functional analysis of New Neurospora crassa Nonself Recognition Loci. Jiuhai Zhao, Charles Hall, Elizabeth Hutchison, David Kowbel, Juliet Welch, N. Louise Glass. Department of Plant and Microbial Biology, University of California, Berkeley USA 94720.

Self/nonself recognition is a ubiquitous and essential function for many organisms. In filamentous fungi, self/nonself recognition is conferred by genetic differences at het (heterokaryon incompatibility) loci. The genes that mediate HI (heterokaryon incompatibility) exhibit characteristic evolutionary signatures, including balancing selection and trans-species polymorphisms. Recent analyses show that genes containing a HET domain are involved in HI, making HET domain genes good candidates for identifying new het loci. In this study, we utilized RNA-seq data from a population of 110 Neurospora crassa strains to look for HET domain genes that were highly polymorphic, have multiple alleles, and show balancing selection, and trans-species polymorphisms. Using this approach, we identified 19 of the 62 HET domain genes in N. crassa that fit the criteria for a het locus. Further, we showed that one of these HET domain genes, NCU09037, functions as a het locus.

722. Combinatorial cationic and oxidative stresses promote the killing of *Candida albicans* cells by human neutrophils. Alistair J P Brown[1], Despoina Kaloriti[1], Mette Jacobsen[1], Zhikang Yin[1], Anna Tillmann[1], Miranda Patterson[2], Deborah A Smith[2], Emily Cook[3], Tao You[4], Iryna Bohovych[1], Celso Grebogi[4], Neil A R Gow[1], Janet Quinn[2], Ken Haynes[3]. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; 2) Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, United Kingdom; 3) School of Biosciences, College of Life & Environmental Sciences, University of Exeter, United Kingdom; 4) Institute for Complex Systems and Mathematical Biology, School of Natural and Computing Sciences, University of Aberdeen, United Kingdom.

Candida albicans is an opportunistic pathogen of humans. It is thought to have evolved as a relatively harmless commensal. *C. albicans* is a frequent cause of mucosal and skin infections (thrush). However, our immune system normally blocks potentially lethal systemic infections of the bloodstream and internal organs. Neutropenic patients are prone to systemic candidiasis because they lack the effective defenses provided by circulating neutrophils. We have shown that the efficient killing of *C. albicans* by human neutrophils is mediated by the potent combinations of stresses they impose on the invading fungus, rather than specific individual stresses. In particular, exposure to the combination of reactive oxygen species and cation fluxes kills *C. albicans* synergistically. We have explored the mechanistic basis for this synergistic killing using genomic exploration and molecular dissection in *C. albicans* combined with dynamic mathematical modeling. Signalling via the Hog1 stress activated protein kinase and the Cap1 AP-1-like transcription factor is inhibited by combinatorial oxidative and cationic stresses, and as a result, their downstream gene targets are not induced. This prevents the activation of normal oxidative and cationic stress adaptation and repair mechanisms. In particular, hydrogen peroxide detoxification mechanisms are inhibited by elevated salt concentrations. This leads to the accumulation of intracellular reactive oxygen species, and ultimately to accelerated necrotic death. Ectopic expression of key detoxification mechanisms in *C. albicans* cells decreases the efficacy of killing by human neutrophils.

723. Phosphoproteomic analysis of the aquatic fungus *Blastocladiella emersonii* during germination. J. Crestani, S. Lopes Gomes. Biochemistry, IQ, USP, Sao Paulo, Brazil.

The aquatic fungus *Blastocladiella emersonii* presents an interesting life cycle with two cell differentiation stages, the germination and the sporulation, during which drastic morphological and biochemical changes are observed. During germination, protein synthesis is inhibited, a transient increase of cAMP levels is observed, activation of PKA is detected, as well as mobilization of cellular glycogen and an efflux of calcium. These results suggest a possible phosphorylation/dephosphorylation control, probably through cell signaling networks, which were not characterized up to now. Therefore, the present work aims to elucidate these signaling mechanisms by using a phosphoproteomic analysis of *B. emersonii* during the germination. Firstly, we compared the phosphoproteomic profile from two distinct cell types of *B. emersonii*, the zoospores and the germling cell (at 45min of germination) by using two-dimensional gels stained with Pro-Q Diamond phosphoprotein dye. The comparison revealed about 82 phosphoproteins from germling cells and 44 phosphoproteins from zoospores. These preliminary results suggest that phosphorylation events may be involved during early germination. To detect the signaling processes and the proteins involved in these events we utilized IMAC-IMAC phosphoproteomic methodology to obtain an enriched phosphopeptide sample from each stage of germination and early vegetative growth (0, 25, 45, 60 and 90 min). Enriched phosphopeptide samples will be detected by using a LTQ Velos Orbitrap mass spectrometer; filtered using DTASelect and analyzed using Ascore and Debunker. The results of this work will contribute to improve the knowledge of the cellular regulatory processes in this early diverging fungus. Financial support: FAPESP and CNPq.

724. Towards an accurate genome: high-throughput proteogenomic validation of *Stagonospora nodorum* genes via sub-cellular proteomics. Kejal Dodhia[1], Robert Syme[1], Thomas Stoll[2], Marcus Hastie[2], James Hane[3], Angela Williams[3], Eiko Furuki[1], Jeffrey Gorman[2], Richard Oliver[1], Kar-Chun Tan[1]. 1) The Australian Centre for Necrotrophic Fungal Pathogens, Environment & Agriculture, Curtin University, Perth, Bentley 6102, Australia; 2) Protein Discovery Centre, Queensland Institute of Medical Research, Herston, Qld 4029, Australia; 3) Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Private Bag No, 5, Wembley WA 6913, Australia.

Stagonospora nodorum is the causal agent of stagonospora nodorum blotch on wheat. *S. nodorum* was the first of the Pleosporales fungi to have its genome sequence published and genes annotated. However, *in silico* gene annotation can be erroneous. Therefore, experimental evidence is often needed to refine gene annotations. Proteogenomics is an emerging high-throughput technique, which is a “direct-to-genome mapping” technique whereby the mass spectra from protein analyses are mapped onto the predicted gene set and/or the 6-frame whole genome translation. In this study, we performed a comprehensive proteogenomic analysis of the secreted, intracellular and cell-wall/membrane sub-proteomes of *S. nodorum* using a two-dimensional liquid chromatography (2D-LC) LTQ Orbitrap MS approach. This study has verified a total of 3580 genes from all sub-proteomes. Of these, 113 had not been experimentally verified previously. When combined with previous proteomic data, 4377 (35% of the total predicted gene set) genes were verified. In addition, all mass spectra were matched to a 6-frame genome translation database to identify evidence of gene model conflicts. The study has found that 2629 genes showed evidence of frame conflicts and extensions of coding exons into annotated introns or untranslated regions. At least 43 potential new genes were identified.

725. Evolutionary Imprint of Fungal PKS-NRPS Catalytic Domains. Daniela Boettger[1], Holger Bergmann[2], Barbara Kühn[1], Ekaterina Shelest[2], Christian Hertweck[1]. 1) Department Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Beutenbergstrasse 11a, 07743 Jena, Germany; 2) Department of Systems Biology/Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Beutenbergstrasse 11a, 07743 Jena, Germany.

Fungal polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) hybrid enzymes produce a broad array of ecologically and medicinally relevant

natural products. To date, only a dozen gene clusters could be matched to the requisite PKS-NRPS pathways and the programming of the multifunctional enzymes is still enigmatic. The (heterologous) expression of chimeras of PKS (lovastatin synthase, LovB) and NRPS (cytochalasin synthetase, CheA) in *Aspergillus terreus* did not result in the production of polyketide-amino acid hybrid molecules and suggests a potential incompatibility of a fungal highly reducing PKS (hrPKS) with the NRPS component of fungal PKS-NRPS hybrids. Furthermore, the heterologous expression of a shortened CheA (truncated after the C domain) in *A. oryzae* did not lead to cyclized products. To rationalize the unexpected outcome of the gene fusion (and shortening) experiments, we accomplished extensive bioinformatic analyses of fungal PKS-NRPS hybrids and LovB-type PKS. Hence, a noncanonical function of *C*-terminal condensation (C) domains in truncated PKS-NRPS homologues and an evolutionary imprint of the PKS-NRPS domains, which reflect the evolutionary history of the entire megasynthase, was inferred. Moreover, the participation of not only the adenylation (A) domain but also the C domain to amino acid selection was shown to be likely. These findings shed new light on the complex code of this emerging class of multifunctional enzymes and will greatly facilitate future combinatorial biosynthesis and pathway engineering approaches towards natural product analogues.

726. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. Jonathan M. Palmer[1], Jeffrey Theisen[1], Rocio Duran[2], Scott Grayburn[2], Ana Calvo[2], Nancy Keller[1]. 1) Medical Microbiology and Immunology, Univ Wisconsin, Madison, WI; 2) Biological Sciences, Northern Illinois University, DeKalb, IL.

Secondary metabolism and development are linked in *Aspergillus* through the conserved regulatory velvet complex composed of VeA, VelB and LaeA. The founding member of the velvet complex, VeA, shuttles between the cytoplasm and nucleus in response to alterations in light. Here we describe a new interaction partner of VeA identified through a reverse genetics screen looking for LaeA-like methyltransferases in *Aspergillus nidulans*. One of the putative LaeA-like methyltransferases identified, LlmF, is a negative regulator of sterigmatocystin production and sexual development. LlmF interacts directly with VeA and the repressive function of LlmF is mediated by influencing the localization of VeA, as over-expression of llmF decreases the nuclear to cytoplasmic ratio of VeA while deletion of *llmF* results in an increased nuclear accumulation of VeA. We show that the methyltransferase domain of LlmF is required for function, however LlmF does not directly methylate VeA *in vitro*. This study identifies a new interaction partner for VeA and highlights the importance of cellular compartmentalization of VeA for regulation of development and secondary metabolism.

727. Overproduction of phleichrome by synthetic inducers and cloning of polyketide synthase genes in phytopathogenic fungus *Cladosporium phlei*. K.-K. So[1], N.-L. Nguyen[1], J.-M. Kim[2], Y.-S. Jang[1], Y.-S Jeong[1], D.-H. Kim[1]. 1) Institute for Molecular Biology and Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea.

Phleichrome pigment produced by a *Cladosporium phlei* is a pathogenic toxin of timothy plant (*Phleum pretense*). Phleichrome reacts with oxygen molecules following light activation to produce highly toxic reactive oxygen species. Phleichrome is structurally similar to elsinochrome and several other 4,9-dihydroxyperylene-3,10-quinone fungal toxins. Phleichrome has a huge potential to be used as photodynamic agent for treatment of cancer and viral infection. Using the UV mutagenesis method we were able to obtain two mutant strains that overproduced phleichrome in different culture conditions compared with the wild type strain. In addition, we synthesized two different diketopiperazines as inducers and confirmed that diketopiperazines significantly enhanced phleichrome biosynthesis in a dose dependent manner. To gain insight into the metabolic pathway of phleichrome production, we performed to clone and sequence several polyketide synthase (PKS) genes. Among the three representative types of PKS, two, one, and one gene for reducing-, partially reducing-, and non-reducing type PKS, respectively, were cloned, sequenced, and characterized. Biological characterization of these genes is underway to determine its role in the production of phleichrome and open the possibility of metabolically engineering this pathway for overproduction of the desired substance.

728. Symbiotic fungal endophytes that confer tolerance for plant growth in saline soil. Zakia Boubakir, Elizabeth Cronin, Susan GW Kaminskyj. Biology, Univ Saskatchewan, Saskatoon, Saskatchewan, Canada.

Fungal endophytes are plant symbionts, and appear to be ubiquitous in plants growing in natural soils. Pioneering work by RS Redman and RJ Rodriguez showed that class II fungal endophytes confer tolerance to harsh growth environments. These endophyte strains are expected to enhance plant growth and improve nutrient uptake under normal and saline conditions, although currently the mechanism(s) is unknown. Soil salinity is one of the most serious agricultural problems that restrict plant growth and crop yield in many areas of the world. Saskatchewan has large areas of salinized soils as well as many saline lakes. In this study, we are characterizing endophyte fungi isolated from salinized soils in southern Saskatchewan. These include potash mine tailings, which are ~ 95 % NaCl. In the spring of 2012 we collected 90 plant samples from 9 sites, from which we isolated ~450 endophyte fungi. Here, we will present a preliminary characterization of isolate Skj422.08. This strain has been shown to confer NaCl tolerance for tomato and wheat that were grown in soil mix watered with fresh water, then stressed with 200 mM or12 g/L NaCl (tomato), or 300 mM or 18g/L NaCl (wheat). Skj422.08 also improved growth when tomato and wheat were grown from seed in 150 mM or 9 g/L NaCl. This project is funded in part by Mosaic Co, a major producer of potash in Saskatchewan, as well as phosphate and micronutrients in other parts of North America, and worldwide.

729. Stable cesium and radiocesium response of Schizophyllum commune. Matthias Gube[1], Alix Günther[2], Flemming Katrin[2], Linde Jörg[3], Raff Johannes[2], Kothe Erika[1]. 1) Microbial Communication, Institute for Microbiology, Friedrich Schiller University of Jena, Thuringia, Germany; 2) Helmholtz-Centre Dresden-Rossendorf, Germany; 3) Hans-Knöll-Institute for Natural Product Research, Jena, Germany.

Radioisotope contamination poses a threat to both ecosystem functioning and public health. Compared with plants, fungi can accumulate much higher amounts of heavy metals and radionuclides in their fruiting bodies. This was seen after the reactor accident of Chernobyl in 1986, when it became clear that fungal uptake of radionuclides such as 60Co, 90Sr, and most importantly 137Cs may reach harmful levels if consumed. It is thus of crucial importance to study radionuclide uptake into fungi to evaluate subsequent migration in the environment, thus allowing for meaningful ecotoxicological risk assessment. Usually, this is being performed by analysing stable isotopes of the same elements, whenever these are available. Due to their lower criticality, this reduces costs and risk of associated experiments. It is generally assumed that the effects of both are identical. However, comparative analyses have seldom been performed, and never with fungi. Ionizing radiation is known to cause effects ranging from DNA and Protein damage to increased oxidative stress and possibly apoptosis in a number of organisms. Especially in gene expression studies, certain reactions such as regulation of protein and nucleic acid repair mechanisms might thus deviate between stable and radioisotope exposure. Thus, differential gene expression of the fully sequenced model organism S. commune was analysed using MACE (Massive Analysis of CDNA Ends) under treatment with stable 133Cs and the b-decaying 137Cs and compared with untreated samples. Differential gene expression analysis points out factors responding to either radiation or Cs ions, thus differentiating between metal ion stress and radiation effects. While several carbohydrate metabolism genes and especially hydrophobin genes are specifically regulated following radiation, most expected responding factors, such as genes involved in stress response or ion and water transport, are

regulated after treatment with both 133Cs and 137Cs.

730. The completion of meiosis in *Ustilago maydis* requires an Ndt80 ortholog. C. E. Doyle[1], H.Y. K. Cheung[1], B. J. Saville[1,2]. 1) Environmental & Life Sciences Graduate Program, DNA Building, Trent University, 2140 East Bank Dr., Peterborough, ON, Canada; 2) Forensic Science Program, DNA building, Trent University, 2140 East Bank Dr. Peterborough, ON, Canada.

Meiosis in the model fungal plant pathogen *Ustilago maydis* requires growth in the plant host; as such, control of meiosis responds to cues received during pathogenic development. To begin investigating this process, an ortholog of the *Saccharomyces cerevisiae* meiotic control protein, Ndt80 (non dityrosine) was identified in *Ustilago maydis*. It was hypothesized to control progression through meiosis and has been designated *mcg1* (meiosis control gene 1). To assess its role in meiosis, *mcg1* deletion mutants were constructed in compatible *U. maydis* haploid strains by replacing the gene with different selectable markers. This allowed the impact of *mcg1* deletion on pathogenesis, teliospore development and the completion of meiosis to be determined. Infections with compatible *Dmcg1* strains were fully pathogenic, but teliospores produced from these crosses displayed a distinctive, abnormal morphology and meiotic segregation assays indicated that they germinated without undergoing meiosis. This suggests that Mcg1 is involved in the regulation of meiosis and teliospore formation. To investigate this possibility that Mcg1 accomplishes this function by acting as a transcription factor, the upstream region of *U. maydis* genes was searched for variants of sites known as middle sporulation elements (MSE, the binding site of *S. cerevisiae* Ndt80). 89 genes with upstream MSEs were screened by RT-PCR, using RNA from dormant teliospores of wild-type and *Dmcg1* strains. The results suggested that transcript levels for 43 of these genes differed in wild-type, relative to *Dmcg1* teliospores, which indicated that the expression of these genes was affected, either directly or indirectly, by Mcg1. Further screens using RT-qPCR allowed the confirmation of genes with increased transcript levels, as well as those with decreased transcript levels, in the *Dmcg1* teliospores relative to the wild-type teliospores. The upstream regions of these genes were screened for the presence of conserved sequence elements. In parallel, Mcg1 was aligned with putative orthologs to identify conserved regions. Based on these alignments, *mcg1* genes containing targeted mutations were synthesized. Together, these analyses begin the determination of how *in planta* transitions in *U. maydis* development are controlled.

731. Impact of changes in the target P450 CYP51 enzyme associated with altered triazole-sensitivity in the Wheat pathogen *Mycosphaerella graminicola*. Eileen Scott[1], Regula Frey[2], Helge Sierotzki[2], Michael Csukai[1]. 1) Syngenta, Biological Sciences, Jealotts' Hill International Research Centre, Bracknell, United Kingdom; 2) Syngenta Crop Protection Munchwilen AG, Research Biology Centre, Schaffhauserstrasse, Stein, Switzerland.

The triazoles are a widely used class of fungicides, targeting the cytochrome P450 sterol 14a-demethylase Cyp51. They are hence also known as the 14a - demethylase inhibitors, the 14-DMIs. Despite heavy use of this chemical class in the field over a considerable period of time, catastrophic resistance has not been observed in the economically important plant pathogen *M. graminicola*. Rather, there has been a slow shift toward reduced sensitivity. A large number of mutations in the Cyp51 gene have been previously associated with this shift in sensitivity to DMIs, although other resistance mechanisms such as alteration in sterol biosynthesis and fungicide uptake and efflux may also play a role. There have been attempts to correlate changes in resistance levels with specific Cyp51 mutations in field isolates. However, due to the genetic diversity of *Mycosphaerella* , the possible effect of non-target site mutations and issues with expression in exogenous fungi making solid conclusions has been problematic.

In order to accurately assess the contribution of each of the target site substitution mutations found in the field associated with 14-DMI resistance we have introduced mutations individually and in combination into the endogenous Cyp51 gene in a uniform genetic background (*M. graminicola* genome sequenced strain, IPO323). Here, we present the findings of the comparative efficacy of varying triazole structures against this comprehensive collection of mutants.

732. Molecular Evolutionary Analysis and Synteny of Fungal GAL Genes. Julien S Gradnigo[1], C. L Anderson[2], R. A Wilson[3], E. N Moriyama[1,4]. 1) School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE; 2) Department of Computer Science and Engineering, University of Nebraska - Lincoln, Lincoln, NE; 3) Department of Plant Pathology, University of Nebraska - Lincoln, Lincoln, NE; 4) Center for Plant Science Innovation, University of Nebraska - Lincoln, Lincoln, NE.

In many fungal species (including Saccharomyces, Candida, Schizosaccharomyces and related genii), genes involved in successive steps of a metabolic pathway are often physically clustered in the genomes. Within genes involved in the Leloir pathway for galactose catabolism, such clustering is considered to facilitate niche adaptation via rapid gene inactivation. This pathway involves three structural genes - GAL1, a galactokinase, GAL7 a uridylyl transferase and GAL10, a bifunctional protein with two epimerase domains. The products of the GAL80, GAL4 and GAL3 genes - a co-repressor, activator and co-activator - cooperatively regulate expression of the structural genes. GAL1 and GAL3 are highly similar (>90% identity) and likely arose from an ancient duplication event. GAL1, 7 and 10 are known to cluster in many divergent fungal lineages, including Saccharomyces, Candida, Schizosaccharomyces, and Cryptococcus. To further investigate potential syntenic patterns in a wider range of fungal lineages including filamentous species, we identified orthologous GAL proteins from over 60 fungi. An initial set of orthologue candidates was generated using a combination of BLAST, reciprocal BLAST and profile hidden Markov model searches. Sequences meeting the percent identity and coverage thresholds established for each protein were subsequently aligned using MAFFT. We then reconstructed maximum likelihood phylogenies and, where necessary, compared predicted secondary structures to produce the orthologue dataset. Location information was obtained from the respective source database (NCBI, JGI or the BROAD Institute). We confirmed that GAL1, GAL7 and GAL10 are not clustered in all 53 species of filamentous fungi we studied. While in 7 species closely related to S. cerevisiae as well as C. albicans, as previously reported, the two GAL10 domains exist as a single fused protein, they exist as separate proteins in Yarrowia lipolytica and not at all in Ashbya gossypii. In all filamentous fungi we examined, these domains exist independently. We found widespread duplication of both domains, and are examining the evolutionary origins of GAL10 proteins and the timing of domain duplication and acquisition events. As GAL10 proteins participate in both the first and final steps of the Leloir pathway, such duplication may promote catabolic efficiency.

733. Meiotic Drive: A Single Gene Conferring Killing and Resistance in Fungal Spore Killer. Pierre Grognet[1,2*], Fabienne Malagnac[1,2], Hervé Lalucque[1,2], Philippe Silar[1,2]. 1) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain, 75205 Paris CEDEX 13 France; 2) Univ Paris Sud, Institut de Génétique et Microbiologie, Bât. 400, 91405 Orsay cedex, France.

Meiotic drives (MD) are nuclear genetic loci ubiquitous in eukaryotic genomes that cheat the Mendel laws by distorting segregation in their favor. All known MD are composed of at least two linked genes, the distorter that acts as a toxin by disrupting the formation of gametes, and the responder that acts as an antitoxin and protects from the deleterious distorter effects. In fungi, MDs are known as Spore Killers (SK). In the model ascomycete *Podospora anserina*, MD has been associated with deleterious effect during ascospore formation of the Het-s prion and in *Neurospora crassa* a resistance gene (responder) to the Sk-2 and Sk-3 distorters has been identified. MDs are easily studied in *P. anserina* thanks to the ascus structure as SKs are identified by the presence of 2-spored asci in crosses between strains. Here, we identify and characterize by targeted deletion in *P. anserina Spok1* and *Spok2*, two MD

elements. We show that they are related genes with both spore-killing distorter and spore-protecting responder activities carried out by the same allele, unlike other known MD. These alleles act as autonomous elements and exert their effects in any region of the genome. Moreover, *Spok1* acts as a resistance factor to *Spok2* killing. As *Spok1* and *Spok2* belong to a multigene family, these Spore Killer genes represent a novel kind of selfish genes that proliferate in population through meiotic distortion.

734. Alkaliphilic fungi from soda lakes and soda soils. Alexey A. Grum-Grzhimaylo[1], Alfons J.M. Debets[1], Marina L. Georgieva[2], Elena N. Bilanenko[2]. 1) Wageningen University, Wageningen, The Netherlands; 2) Lomonosov Moscow State University, Moscow, Russia.

Filamentous fungi growing optimally at pH exceeding neutral values have received little scientific attention and generally it is believed that only prokaryotic organisms are able to survive harshly elevated ambient pH values. To date, only a handful of alkaliphilic fungi (i.e. fungi growing optimally at pH > 9) have been reported. The few studies devoted to fungi growing at high pH lack a systematic molecular phylogenetic analysis. Our study aims to reveal the taxonomic distribution of alkaliphilic filamentous fungi we isolated from soils at different sites near soda lakes. We intend to test if the alkaliphilic trait has occurred independently throughout the fungal kingdom or rather once in a single lineage. Soda lakes and soils with pH ranging from 8 to as high as 11 are believed to be the natural habitats for alkaliphiles. The high pH is maintained mainly due to strong buffering capacity of carbonate salts presented there. We used alkaline agar medium (the pH is buffered at around 10) with antibiotic as a selective medium for screening for potential alkaliphilic fungi in the collected soil samples. By these means we isolated 99 ascomycetous strains which were capable of growing, to different extents, at pH 10. Two thirds of the total number turned out to be anamorphic fungi displaying only asexual sporulation (mostly *Acremonium*-like) while only 19 strains were holomorphic homothallic being able to develop the full life cycle. Seventeen isolates produced only sterile mycelium without reproductive structures under laboratory conditions. We sequenced five genes (SSU rDNA, LSU rDNA, RPB2, TEF1alpha, ITS region of rDNA) to pinpoint the taxonomic positions of all isolated. After phylogenetic reconstructions all our strains had tendency to group in two different lineages within the *Ascomycota*. The first lineage is the *Plectosphaerellaceae* family (insertae sedis within subphylum *Hypocreomycetidae*) which harbors 39 alkaliphilic isolates while the second lineage is *Emericellopsis*-clade (order *Hypocreales*, subphylum *Hypocreomycetidae*) within the *Acremonium* cluster containing 30 strains. The remaining 30 isolates are presumably alkalitolerant members of the *Pleosporinae* and *Sordariales* lineages known to be ubiquitous soil fungi.

735. A new method for gene mining and enzyme discovery. Y. Huang[1,2,3], P. Busk[1], M. Grell[1], H. Zhao[2,3], L. Lange[1]. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610041, PR China; 3) University of the Chinese Academy of Sciences, Beijing 100049, PR China.

Peptide pattern recognition (PPR) is a non-alignment based sequence analysis principle and methodological approach, which can simultaneously compare multiple sequences and find characteristic features. This method has improved the understanding of structure/function relationship for enzymes within the CAZY families, which would make it easier to predict the potential function of novel enzymes, creating bigger promises for industrial purposes. *Mucor circinelloides*, member of the former subdivision Zygomycota, can utilize complex polysaccharides such as wheat bran, corncob, xylan, CMC and avicel as substrate to produce plant cell wall degrading enzymes. Although the genome of *M. circinelloides* has been sequenced, only few plant cell wall degrading enzymes are annotated in this species. In the present project, PPR was applied to analyze glycoside hydrolase families (GH family) and mining for new GH genes in *M. circinellolides* genome. We found 19 different genes encoding GH3, GH5, GH6, GH7, GH9, GH16, GH38, GH43, GH47 and GH125 in the genome. Of the three GH3 encoding genes found, one was predicted by PPR to encode a b-glucosidase. We expressed this gene in *Pichia pastoris* and found that the recombinant protein has high b-glucosidase activity (4884 U/mL). In this work, PPR provided targeted short cut to discovery of enzymes with a specific activity. Not only could PPR pinpoint genes belonging to different GH families but it did also predict the enzymatic function of the genes.

736. Occurrence of dsRNA mycovirus (LeV-FMRI 2427) in edible mushroom *Lentinula edodes* and its meiotic stability. J.-M. Kim[1], S.-H. Yun[2], M.-S Yang[2], D.-H. Kim[2]. 1) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea; 2) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea.

The dsRNA was first found in the malformed cultures of *Lentinula edodes* strain FMRI 2427, one of three most popular sawdust cultivating commercial strains of shiitake. This dsRNA was also found in the healthy-looking fruiting bodies and actively growing mycelia. Cloning of partial genome of dsRNA revealed the presence of RdRp sequence of a novel *L. edodes* mycovirus (LeV) and sequence comparison of the clone amplicon showed the identical sequence to the known RdRp genes of LeV found in strain HKA. Meiotic stability of dsRNA was examined by the measuring the ratio of the presence of dsRNA among the sexual monokaryotic progenies. More than 40% of monokaryotic progenies still contained the dsRNA indicating the persistence of dsRNA during sexual reproduction. Comparing mycelial growth of monokaryotic progenies suggested that, although variations in growth rate existed among progenies, there appears no direct relationship of mycovirus infection to the growth rate.

737. Analysis of fungal communities associated with grapevine wood diseases, based on fungal ITS pyrosequencing. Nicolas Lapalu[1,2], Angélique Gautier[2], Laetitia Brigitte[1,2], Jessica Vallance[3], Emilie Bruez[3], Joelle Amselem[1,2], Hadi Quesneville[1], Valérie Laval[2], Marc-Henri Lebrun[2], Patrice Rey[3]. 1) INRA-URGI, Versailles, France; 2) INRA, BIOGER, Thiverval Grignon, France; 3) INRA, Santé Végétal, Bordeaux, France.

The Grapevine Trunk Diseases (GTDs) are the most common diseases of grapevine wood inducing a slow decay leading to plant death. Due to the environmental impact, chemical treatments are no longer authorized, and prevention or trunk removal are the last available control methods. Fighting against these slow evolving diseases requires a better knowledge of fungal and bacterial communities associated with GTDs. Our approach is based on fungal species identification using ITS (Internal Transcribed Spacer) sequences obtained by pyrosequencing (Roche 454) of grapevine wood samples. DNAs were extracted from different parts of grapevine trunks and amplified using fungal specific ITS primers. A workflow was set up to analyze pyrosequencing data, allowing taxonomic assignment with a database extracted from Genbank and curated with the FungalITSextractor (Nilsson H et al. 2010). The pipeline links tools, including cleaning and extracting ITS sequences to limit the impacts of sequencing errors on clustering and assignation steps. Then, Operational Taxonomic Units (OTUs) detection and taxonomic assignments were performed with the QIIME package (Caporaso JG et al. 2010). Samples from different vineyards (infected or not), with several dates of sampling, were analyzed. Different ITS PCR primers and technical replicates were tested using controls corresponding to the mixture of fungal DNAs from diverse known species. These controls highlighted interests and limits of PCR amplicons pyrosequencing and the relevance of the bioinformatics methods to extract accurate data to fit to the context of taxonomy.

738. The Antidepressant Sertraline Provides a Promising Therapeutic Option for Neurotropic Cryptococcal Infections. Bing Zhai, Cheng Wu, Linqi Wang, Matthew Sachs, Xiaorong Lin. Biology, Texas A&M University, TAMU-3258, TX.

Therapeutic treatment for systemic mycoses is severely hampered by the extremely limited number of antifungals. Treatment for fungal infections in the

central nervous system is further compounded by the poor CNS penetration of most antifungals due to the blood-brain barrier. Only a few fungistatic azole drugs, such as fluconazole, show reasonable CNS penetration. Here we demonstrate that sertraline (brand name Zoloft), the most prescribed antidepressant, displays potent antifungal activity against Cryptococcus neoformans, the major causative agent of fungal meningitis. In the in vitro assays, this neurotrophic drug is fungicidal to all natural Cryptococcus isolates tested at clinically relevant concentrations. Furthermore, sertraline interacts synergistically or additively with fluconazole against Cryptococcus. Importantly, consistent with our in vitro observations, sertraline alone reduces the brain fungal burden at a comparable efficacy as fluconazole in a murine model of systemic cryptococcosis. It works synergistically with fluconazole in reducing the fungal burden in brain, kidney, and spleen. In contrast to its potency against Cryptococcus, sertraline is less effective against strains of Candida species and its interactions with fluconazole against Candida strains are often antagonistic. Therefore, our data suggest the unique application of sertraline against cryptococcosis. To understand the antifungal mechanisms of sertraline, we screened the whole genome deletion collection of Saccharomyces cerevisiae for altered sertraline susceptibility. Gene ontology analyses of selected mutations suggest that sertraline perturbs translation. In vitro translation assays using fungal cell extracts show that sertraline inhibits protein synthesis. Taken together, our findings indicate the potential of adopting this antidepressant in treating cryptococcal meningitis.

739. Analysis of gene expression of proteases of *Trichoderma* spp during confrontation with plant pathogens in vivo. Valdirene Monteiro[1], Eva Couto[1], Fenix Oliveira[1], Marcela Suriani[2], Raphaela Georg[2], Cirano Ulhoa[2]. 1) Universidade Estadual de Goiás, Anápois, Brazil; 2) Universidade Federal de Goiás, Goiânia, Brazil.

The genus *Trichoderma* with teleomorfismo in Hypocrea comprises a group of saprophytic fungi and micoparasitas widely used as biological control agents of soil borne plant pathogens. Among the mechanisms proposed for the biocontrol of *Trichoderma* species are mycoparasitism by production of cell wall degrading enzymes of plant pathogens, antibiotic production volatile and non-volatile competition for nutrients, rhizosphere competent and induction of defense responses in plants. In recent years, several efforts have been made to better understanding of molecules involved in mycoparasitism. In this study we assessed the expression of protease genes of *Trichoderma* species by qRT-PCR and the gene for alpha-tubulin as normalizer. Differential expression was assessed from antagonism of *Trichoderma virens* and *Trichoderma harzianum* during contact and after contact with the host hyphae of fungal pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The "primers" were designed based on the sequences of the genes encoding these enzymes deposited in the database DOE Joint Genome Institute (http://www.jgi.doe.gov/). The protease genes of both species were aspartate protease, Carboxypeptidase, cysteine protease, Subtilisin peptidase, trypsin, protease Serino metallopeptidase. Initial results showed that during the contact of *Trichoderma virens* and *Rhizoctania solani* for metalloprotease gene is 80% higher than that expressed in the same condition for *Trichoderma harzianum*. When observing the condition after contact between *T.virens* and *Sclerotinia sclerotiorum* the metalloprotease gene does not have its expression as required presenting least 60% expressed in relation to genes aspartate, carboxy, and subtilisin serine proteases. The expression was very different between the clashes of *Trichoderma* and plant pathogens as well as for contact times. Leading us to believe that specific classes of proteases are required at different stages of contact of *Trichoderma* and pathogen. Person's test was performed to analyze the correlation between the expression of these genes and other genes of proteases involved in mycoparasitism as glucanases and chitinases.

740. Analysis of differential protein profile during antagonism of *Trichoderma harzianum* and *Sclerotinia sclerotiorum*. Valdirene Monteiro[1], Roberto Silva[3], Cirano Ulhoa[2]. 1) Universidade Estadual de Goiás, Anápois, Anápolis, Brazil; 2) Universidade Federal de Goiás, Goiânia, Brazil; 3) University of São Paulo.

Trichoderma sp. are effective biocontrol agents for several soil-borne fungal plant pathogens including *Fusarium* sp., *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* some species are also known for their abilities to enhance systemic resistance to plant diseases as well as overall plant growth. The biocontrol exercised by *Trichoderma* can occur by means of several antagonistic mechanisms such as nutrient competition, antibiotic production and mycoparasitism. Mycoparasitism has been proposed as the major antagonistic mechanism displayed by *Trichoderma* sp. In the present study were examined the differential production of proteins in three different conditions were antagonism. These conditions were *T. harzianum* vs mycelium of fungal pathogens *Sclerotinia sclerotiorum*; *Trichoderma harzianum* vs esclerotia of fungal pathogens *Sclerotinia sclerotiorum* and *Trichoderma harzianum* vs leaves beans infected with *Sclerotinia sclerotiorum*. The differential expression of proteins in different conditions of antagonism was analyzed by two-dimensional difference gel electrophoresis (2D-DIGE). Initial analyzes showed profiles varied between interactions. The proteins which were expressed were so different and trypsin-like SM1 between conditions *T.harzianum* and bean leaves and *T. harzianum* and mycelium. There is a higher expression of glucanases and chitinases in the condition of the interaction between *T. harzianum* and sclerotia. It was observed the presence of three proteins predicted molecular weight of 15kDa that are present in all tested conditions but with different expression levels. These and other proteins are in phase sequencing.

741. Effector proteins in fungal defense against fungivorous nematodes: Diversity and regulation of expression. D.F. Plaza[1], S.S. Schmieder[1], C.E. Stanley[2], S. Bleuler-Martinez[1], A.J. deMello[2], M. Aebi[1], M. Künzler[1]. 1) Institute of Microbiology, ETH Zürich, Wolfgang-Pauli-Str.10, 8093 Zürich; 2) Institute for Chemical and Bioengineering, ETH Zürich, Wolfgang-Pauli-Str.10, 8093 Zürich.

The regulation of defense mechanisms of fungi is poorly understood. So far, research has focused mainly on the regulation and production of toxic secondary metabolites and peptides. Several lines of evidence also implicate cytoplasmic lectins, many of which are up-regulated during fruiting body development, in fungal defense. We could demonstrate that many of these lectins are toxic, not only to model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster*, but also to fungivorous nematodes. In order to gain more insight into the diversity and regulation of the defense response of fungi against predatory nematodes, we studied the genome-wide gene expression in the model organism *Coprinopsis cinerea* upon challenge with the fungivorous nematodes *Aphelenchus avenae* and *Bursaphelenchus willibaldi*, using next generation RNA-seq. Preliminary transcriptome data shows that the sets of upregulated genes differ between the two nematodes tested, suggesting a nematode-specific defense response. Whereas *B. willibaldi* induced the expression of genes hypothetically involved in secondary metabolite production, such as a non-ribosomal peptide synthase and a terpenoid synthase; *A. avenae* induced previously characterized nematotoxic fungal lectins, such as CGL2, CCL1 and a chimeric RicinB-fold protein. To characterize this inducible defense response in more detail, we developed a reporter system in *C. cinerea* consisting of the *cgl2* promoter coupled to the gene encoding the red fluorescent protein dTomato. The reporter *C. cinerea* strain was grown into a microfluidics device with compartmentalized channels and confronted with *A. avenae*. This system allowed us to microscopically monitor the induction of the effector molecule within the mycelium over time. Preliminary results indicate that the induction can spread within hyphae but is locally confined. The expression of the reporter protein dTomato, driven by the *cgl2* promoter, was first detected after 10 h of nematode predation. In conclusion, our findings show, first, that the defense of *C. cinerea* against fungivorous nematodes is regulated at the level of transcription. Second, we could demonstrate that the transcriptional induction of defense effector proteins in response to predation is not only locally confined but also predator-specific.

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AUTHOR LIST

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W

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AUTHOR LIST

Z

LIST OF PARTICIPANTS

Duur K. Aanen
Wageningen University
Phone: 0031-317-483144
duur.aanen@wur.nl

Salma Abdeljalil
Centre of Biotechnology of Sfax
Phone: 21622731918
salma.abdeljalil@gmail.com

Gerard C Adams Jr
Univ of Nebraska-Lincoln
Phone: 402-472-2858
gadams3@unl.edu

Kate Juliette Affeldt
University of Wisconsin-Madison
Phone: (608) 262-1958
kjschmitt2@wisc.edu

Oleg Agafonov
University of Stavanger
Phone: (47)40340499
oleg.agafonov@uis.no

Pablo S Aguilar
Institut Pasteur de Montevideo
Phone: +598 2522 0910
aguilar@pasteur.edu.uy

Jesus L Aguirre
I de Fisiologia Celular-UNAM
Phone: 5255 5622-5651
jaguirre@ifc.unam.mx

Audrey M V Ah-Fong
Univ California, Riverside
Phone: (951) 827-3932
audreya@ucr.edu

Steven Ahrendt
University of California, Riverside
Phone: (951) 827-2363
sahre001@ucr.edu

Md. Kausar Alam
University of Saskatchewan
Phone: 306 341 2765
kausar.alam@usask.ca

Sabine E Albermann
WWU Muenster
Phone: 4.9251832499e+011
sabine.albermann@wwu.de

Noam Alkan
Weizmann Institute of Science
Phone: 972-8-9342739
noam.alkan@weizmann.ac.il

Fausto Almeida
University of São Paulo
Phone: +55(16)36023066
fbralmeida@usp.br

Joelle Amselem
INRA
Phone: 33 1-30-833395
joelle.amselem@versailles.inra.fr

George E Anasontzis
Chalmers University of Technology
Phone: 46733603904
george.anasontzis@chalmers.se

Mikael R Andersen
Department of Systems Biology
Phone: 4545252675
mr@bio.dtu.dk

James B Anderson
Univ Toronto
Phone: (905) 828-5362
janderso@utm.utoronto.ca

Jennifer L Anderson
Uppsala University, EBC
Phone: +46 018-471 2827
jennifer.anderson@ebc.uu.se

Jonathan P Anderson
CSIRO
Phone: +61 8 93336103
jonathan.anderson@csiro.au

Matthew Z Anderson
University of Minnesota
Phone: 408-394-0489
mzanders@umn.edu

Karl-Magnus Andersson
Lund University
Phone: 46730377338
karl-magnus.andersson@biol.lu.se

Alex Andrianopoulos
University of Melbourne
Phone: 61 3-9344-5164
alex.a@unimelb.edu.au

Caroline Angelard
University of Lausanne
Phone: 41786714773
caroline.angelard@unil.ch

Sarah L Anglin
Millsaps Col
Phone: (601) 974-1414
mcguisl@millsaps.edu

Amanda C.C. Antoniêto
University of São Paulo
Phone: (16) 81004410
amandaantonieto@yahoo.com.br

Diana Anyaogu
Technical University of Denmark
Phone: +45 45252500
dca@bio.dtu.dk

Kahandawa Ariyawansa
Massey University
Phone: 6421 1435681
sameera.ariyawansa@agresearch.co.nz

Jose Arnau
Novozymes
Phone: +45 44461218
joau@novozymes.com

Martha B Arnaud
Stanford University School of Medicine
Phone: (415) 602-7488
arnaudm@stanford.edu

A. Elizabeth Arnold
The University of Arizona
Phone: 5206217212
arnold@ag.arizona.edu

Nina K Aro
VTT Technical Research Centre
Phone: 3.5850356463e+011
nina.aro@vtt.fi

Felipe R Arredondo
Oregon State University
Phone: (541)737-3679
felipe.arredondo@oregonstate.edu

Fred O Asiegbu
University of Helsinki
Phone: +358 9 191 58109
Fred.Asiegbu@Helsinki.fi

Lea Atanasova
Vienna University of Technology
Phone: 4.3676583722e+011
lea.atanasova@tuwien.ac.at

Sara J Atkinson
Coker College
Phone: (843) 340-3255
sara.atkinson@coker.edu

Jin-Ho Baek
Chonbuk National Univ.
Phone: 82 10 2728 3600
sadasoul@nate.com

Yong-Sun Bahn
Yonsei University
Phone: -7601
ysbahn@yonsei.ac.kr

Scott E Baker
Pacific Northwest Natl Lab
Phone: (509) 372-4759
scott.baker@pnnl.gov

Clara Baldin
Hans-Knoell-Institute
Phone: +49 (0)3641 532-1382
clara.baldin@hki-jena.de

Thomas Baldwin
Univ of Georgia
Phone: 518-322-9435
tbaldwin@uga.edu

Dithi Banerjee
University at Buffalo, SUNY
Phone: 7168292091
dithiban@buffalo.edu

Nabaraj Banjara
University of Nebraska-Lincoln
Phone: 402-472-2825
nabaraj.banjara@huskers.unl.edu

Flora Banuett
California State University
Phone: (562)-985-5535
fbanuett@csulb.edu

Sharief Barends
DuPont IB
Phone: 31715686159
sharief.barends@dupont.com

Bridget M Barker
Montana State Univ
Phone: 406-994-7467
bridget.barker@montana.edu

Kerrie W Barry
DOE Joint Genome Institute
Phone: (925) 296-5672
kwbarry@lbl.gov

Salomon Bartnicki-Garcia
CICESE
Phone: 52 646-175-0590
bart@citrus.ucr.edu

Evelina Y. Basenko
University of Georgia
Phone: (706) 542-1550
ebasenko@uga.edu

Sebastian Baumann
Institute for Microbiology
Phone: 0049 (0)211 8114834
baumann@hhu.de

Oezguer Bayram
Inst Microbiology & Genetics
Phone: 49 551 39 37 72
obayram@gwdg.de

Hoda Bazafkan
AIT Austrian Institute of Techno
Phone: 0043 664 88390594
karin.schlaudoschich@ait.ac.at

Lasse Bech
Aalborg University
Phone: 4522431410
lab@bio.aau.dk

Nicola Beckmann
Biocenter, Innsbruck Med. Uni.
Phone: 4.3650525009e+011
nicola.beckmann@i-med.ac.at

Maria-Soledad Benitez
Duke University
Phone: 9196607362
maria.benitez.ponce@duke.edu

Joan W Bennett
Rutgers University
Phone: 848-932-6223
profmycogirl@yahoo.com

Richard Bennett
Brown University
Phone: (401) 863-3121
richard_bennett@brown.edu

Calmes Benoit
University of Angers
Phone: 689424176
calmes.b@gmail.com

Isabelle Benoit
CBS-KNAW
Phone: +31 (0)30 2122621
i.benoit@cbs.knaw.nl

Johan Philipp Benz
UC Berkeley
Phone: (510) 666-2556
ph.benz@berkeley.edu

Mary L Berbee
Univ British Columbia
Phone: (604) 822-3780
mary.berbee@gmail.com

LIST OF PARTICIPANTS

Leslie G Beresford
Novozymes Inc.
Phone: (530) 757-8100 4627
LSBF@novozymes.com

Randy M Berka
Novozymes, Inc
Phone: (530) 757-4974
ramb@novozymes.com

Judith G Berman
Tel Aviv University
Phone: 03-640-7633
jberman@post.tau.ac.il

Daniel Berry
Massey University
Phone: 6463505543
d.berry@massey.ac.nz

Maria Célia Bertolini
Institute of Chemistry
Phone: 55-16-33019675
mcbertol@iq.unesp.br

Margherita Bertuzzi
Imperial College London
Phone: 020 7594 7409
margherita.bertuzzi06@imperial.ac.uk

Sinem Beyhan
University of California, San Francisco
Phone: (415) 502-4810
sinem.beyhan@ucsf.edu

Monica Bhaskar
Helsinki University
Phone: 3.5844530354e+011
monica.bhaskar@helsinki.fi

Michael J Bidochka
Brock University
Phone: 905-688-5550 x3392
bidochka@brocku.ca

Elaine M Bignell
Imperial College London
Phone: 00 44 207 594 2074
e.bignell@imperial.ac.uk

Robert Blake Billmyre
Duke University
Phone: 4438040705
bbillmyre@gmail.com

Ulrike Binder
Medical University Innsbruck
Phone: -80220
ulrike.binder@i-med.ac.at

Vasileios Bitas
Pennsylvania State Univ
Phone: (814) 441-0690
vzb107@psu.edu

Sandra Bloemendal
Ruhr-University Bochum
Phone: 4.9234322247e+011
sandra.bloemendal@rub.de

Sara J Blosser
Montana State University
Phone: (406) 994-7468
sara.wezensky@msu.montana.edu

Burton H Bluhm
University of Arkansas
Phone: (479)575-2677
bbluhm@uark.edu

Marike J. Boenisch
Biocenter Flein Flottbek
Phone: -43105
marike.boenisch@uni-hamburg.de

Daniela Boettger
Hans-Knöll-Institute
Phone: 1.1491765511e+014
daniela.boettger@hki-jena.de

Michael Bolker
Philipps University
Phone: 49 6421-2821536
boelker@staff.uni-marburg.de

Stephanie R Bollmann
Oregon State University
Phone: (541)737-3679
stephanie.bollmann@oregonstate.edu

Melvin D Bolton
USDA - ARS
Phone: 701 239 1373
melvin.bolton@ars.usda.gov

Gregory Bonito
Duke University
Phone: 9196607363
gmb2@duke.edu

Katherine A Borkovich
University of California
Phone: (951) 827-2753
katherine.borkovich@ucr.edu

Joerg Bormann
University Hamburg
Phone: 4.9404281631e+011
bormannj@botanik.uni-hamburg.de

Indrani Bose
Western Carolina University
Phone: (828) 227-3658
ibose@email.wcu.edu

Jordi C Boshoven
Wageningen University
Phone: 31613970288
jordi.boshoven@wur.nl

Ourdia Bouzid
Utrecht Univ
Phone: 31 030 2122600
o.bouzid@uu.nl

Joanna K Bowen
Plant & Food Research
Phone: +64 9 925 7154
joanna.bowen@plantandfood.co.nz

Barry J Bowman
University of California
Phone: (831) 459-2245
bbowman@ucsc.edu

Emma-Jean Bowman
Univ California
Phone: (831) 459-3448
rbowman@biology.ucsc.edu

Axel A Brakhage
Leibniz Institute Infection Biol
Phone: 49 3641-5321000
axel.brakhage@hki-jena.de

Sara Branco
University of California
Phone: 510 688 8583
sara.mayer.branco@gmail.com

Alexandra C Brand
Univ Aberdeen
Phone: 44 1224-555878
a.brand@abdn.ac.uk

Christoph Braun
Bayer SAS - Bayer CropScience
Phone: 33 472 85 23 43
christophandreas.braun@bayer.com

Gerhard H Braus
Georg-August Univ
Phone: 49 551-39-3771
gbraus@gwdg.de

Susanna A Braus-Stromeyer
Georg-August-Univ Goetti
Phone: (0551) 393817
sbraus@gwdg.de

Gustavo Adolfo Bravo Ruiz
University of Cordoba
Phone: 670472051
b12brrug@uco.es

Erin L Bredeweg
Oregon State Univ
Phone: (541) 737-4399
erin.bredeweg@gmail.com

Marin Brewer
University of Georgia
Phone: 7065421254
mtbrewer@uga.edu

Susan M Brewer
DuPont Industrial Biosciences
Phone: 650-846-4632
susan.brewer@dupont.com

Matthias Brock
Friedrich Schiller University and Hans Knoell Inst
Phone: 4.9364153217e+012
matthias.brock@hki-jena.de

Stuart Brody
Univ California, San Diego
Phone: (858) 534-2619
sbrody@ucsd.edu

Alistair J P Brown
University of Aberdeen
Phone: +44 1224 437482
al.brown@abdn.ac.uk

Daren W Brown
USDA/ARS
Phone: (309) 681-6230
browndw@ncaur.usda.gov

Jessica C Brown
U. California, San Francisco
Phone: (617) 258-5190
jessica.brown@ucsf.edu

Steve Brown
Novozymes Inc
Phone: (530) 757-8104
shbr@novozymes.com

Michael Brunner
Univ Heidelberg
Phone: 49 6221-544207
michael.brunner@bzh.uni-heidelberg.de

Tom D Bruns
Univ California, Berkeley
Phone: (510) 642-7987
pogon@berkeley.edu

Jerzy Brzywczy
Institute of Biochemistry &Bioph
Phone: -5921291
jurek@ibb.waw.pl

Joanna A Bueche
UC Berkeley
Phone: 510-643-2546
jbueche@berkeley.edu

Hayley E Bugeja
University of Melbourne
Phone: +61 411 409 949
h.bugeja@unimelb.edu.au

Vincent Bulone
Royal Inst Biotech (KTH)
Phone: 46 70 389 1122
bulone@kth.se

Kathleen M Burchhardt
North Carolina State University
Phone: 919-513-4840
kmpitche@ncsu.edu

Maria A. Burggraaf
Institute of Biology Leiden
Phone: +31(0)71 5274745
m.a.van.welzen@biology.leidenuniv.nl

Claire Burns
Washington and Jefferson College
Phone: (812) 219-6499
burnsc@indiana.edu

Kathryn E Bushley
Oregon State Univ
Phone: (541) 908-0116
bushleyk@science.oregonstate.edu

Geraldine Butler
Univ Col Dublin
Phone: 353 1-7166885
gbutler@ucd.ie

Nicole Bühler
Karlsruhe Institut of Technology
Phone: 0721-60844633
Nicole.Buehler@kit.edu

Julia Böhm
Ruhr-University Bochum
Phone: 4.9234322566e+011
julia.boehm@rub.de

Ilva Esther Cabrera
Univ of California, Riverside
Phone: (951) 827-3190
ilva.cabrera@email.ucr.edu

LIST OF PARTICIPANTS

Mark X Caddick
Univ Liverpool
Phone: 44 151-795-4566
caddick@liv.ac.uk

Marie-Cecile Caillaud
The Sainsbury Laboratory
Phone: +44(0)1603450430
marie-cecile.caillaud@tsl.ac.uk

Silvia Calo Varela
DUKE University Medical Center
Phone: 919.684.2809
silvia.calovarela@duke.edu

Ana M Calvo-Byrd
Northern Illinois University
Phone: (815) 753-0451
amcalvo@niu.edu

Leona Campbell
Univ Sydney
Phone: 61293513758
leona.campbell@sydney.edu.au

Thiago S Candido
UNESP - IQ - Araraquara
Phone: (55) (14) 96057215
thiago.s.candido@gmail.com

Paulo Francisco Canessa
Universidad Catolica de Chile
Phone: 5626862348
paulo.canessa@gmail.com

Nallely Cano-Dominguez
CICESE
Phone: 01 (646) 175 05 00
ncanodominguez@gmail.com

David Canovas
Univ de Sevilla
Phone: 34 954 55 59 47
davidc@us.es

Ignazio Carbone
North Carolina State University
Phone: (919) 513-4866
ignazio_carbone@ncsu.edu

Nohemi Carreras-Villasenor
LANGEBIO-Cinvestav
Phone: 52 462 1527287
ncarreras@ira.cinvestav.mx

Dee A Carter
Univ Sydney
Phone: 61 29351-5383
dee.carter@sydney.edu.au

Gemma M Cartwright
Massey Univ
Phone: 64 63505515
g.m.cartwright@massey.ac.nz

Jeffrey W Cary
ARS-SRRC
Phone: 504-286-4264
Jeff.Cary@ars.usda.gov

Sonia M Castanheira
CNB-CSIC
Phone: +34 923294912
smcastanheira@cnb.csic.es

MARTA CASTRILLO JIMÉNEZ
University of Seville
Phone: +34 954 555 948
martacastrillo@us.es

Ernestina Castro-Longoria
CICESE
Phone: 646 1750500
ecastro@cicese.mx

David E A Catcheside
Flinders Univ
Phone: 618 8201-2335
david.catcheside@flinders.edu.au

Gustavo C Cerqueira
Broad Institute
Phone: 2405155696
gustavo@broadinstitute.org

François Cesbron
Heidelberg Biochemistry Centre
Phone: -8412261
cesbron.francois@gmail.com

Nadia Chacko
Texas A&M University
Phone: (979) 845-7259
nchacko@bio.tamu.edu

Jimmy Chan
DuPont Industrial Biosciences
Phone: 6166179146
jimmy.chan@dupont.com

Chia-Chen Chang
University of Edinburgh
Phone: +44(0)131 651 3337
jeanwhale@gmail.com

Jinhui Chang
The Chinese University of Hong Kong
Phone: (852)39431331
tjchang@cuhk.edu.hk

Pranatchareeya Chankhamjon
HKI
Phone: +49 (0)3641 532 1392
pranatchareeya.chankhamjon@hki-jena.de

Nikki D Charlton
The Samuel Roberts Noble Foundation
Phone: 580-224-6964
ndcharlton@noble.org

Biju V Chellappan
University of Amsterdam, SILS
Phone: 31687098694
b.chellappan@uva.nl

Julian J-L Chen
Arizona State University
Phone: 480-965-3650
JLChen@asu.edu

Weidong Chen
USDA ARS
Phone: 5093359178
w-chen@wsu.edu

Yuan Chen
Duke University Medical Center
Phone: 9193217870
yuan.chen@duke.edu

Xuanjin Cheng
The Chinese University of Hong Kong
Phone: (852)39431331
jeanch12345@gmail.com

Helene Chiapello
CR INRA Toulouse Midi-Pyrenees
Phone: 33 5 61 28 52 87
helene.chiapello@toulouse.inra.fr

Yangrae Cho
University of Hawaii at Manoa
Phone: 808-956-5305
yangrae@hawaii.edu

Jaehyuk Choi
Seoul National University
Phone: -5484
jaehyukc@gmail.com

Jaeyoung Choi
Seoul National University
Phone: -5746
nizam84@snu.ac.kr

Tetsuya Chujo
Massey University
Phone: -9397
t.chujo@massey.ac.nz

Hyunjung Chung
Seoul National University
Phone: -5484
comcato@snu.ac.kr

Alice C L Churchill
Cornell Univ
Phone: (607) 255-0872
acc7@cornell.edu

Lynda M Ciuffetti
Oregon State Univ
Phone: (541) 737-2188
ciuffetl@science.oregonstate.edu

Pierre-Henri J Clergeot
Stockholms Universitet
Phone: 46 8 16 37 56
clergeot@botan.su.se

Jeffrey J Coleman
Rhode Island Hosp/Alpert Medical School of Brown University
Phone: (401) 444-7309
jjcoleman@partners.org

Jérôme Collemare
Wageningen University
Phone: (+31) 0317-483096
jerome.collemare@wur.nl

Bradford J Condon
Cornell Univ
Phone: (607) 255-3200
bjc225@cornell.edu

Michael R Cooper
University of Louisville
Phone: 5023381405
michael.cooper@louisville.edu

Evelyne Coppin
Univ Paris - Sud
Phone: 33 169 157012
evelyne.coppin@igmors.u-psud.fr

Samuel T Coradetti
University of California
Phone: 510-666-2559
scorad.register@gmail.com

Padraic G Corcoran
Uppsala University
Phone: 735853162
padraic.corcoran@ebc.uu.se

Ben J.C. Cornelissen
University of Amsterdam
Phone: 0031 20 5257707
b.j.c.cornelissen@uva.nl

Nicolas Corradi
University of Ottawa
Phone: +1 6132529432
ncorradi@uottawa.ca

Cristina Corral Ramos
University of Cordoba
Phone: 34665882311
z12corac@uco.es

Luis M Corrochano
University of Seville
Phone: 34 954 550 919
corrochano@us.es

Peter J Cotty
University Arizona
Phone: (520) 626-5049
pjcotty@email.arizona.edu

Mikael S Courbot
Syngenta Crop Protection Munchwilen
Phone: 41628660368
mikael.courbot@syngenta.com

Sarah F Covert
Univ Georgia
Phone: (706) 542-1385
covert@uga.edu

Robert A Cramer
Geisel School of Medicine at Dartmouth
Phone: (603) 650-1040
robert.a.cramer.jr@dartmouth.edu

Rhaisa Crespo
Univ of Puerto Rico Mayaguez
Phone: 787-614-7278
rhaisa.crespo@upr.edu

Juliana Crestani
USP
Phone: 5.5129730841e+011
julicrestani@gmail.com

Daniel Croll
ETH Zurich
Phone: +41 44 632 3387
daniel.croll@me.com

Michael Csukai
Syngenta
Phone: +44 1344 414094
michael.csukai@syngenta.com

Fernanda B Cupertino
Instituto de Quimica,UNESP
Phone: 55-16-33019804
fernanda_cupertino@yahoo.com.br

LIST OF PARTICIPANTS

Tim A Dahlmann
Ruhr-University Bochum
Phone: 4.9234322221e+011
tim.dahlmann@rub.de

Pascale Daran-Lapujade
Delft Univ. of Technology
Phone: +31 15 2789965
p.a.s.daran-lapujade@tudelft.nl

Heather L Darbyshir
University of Nottingham
Phone: +44 115 951 3203
sbxhd4@nottingham.ac.uk

Asen I Daskalov
IBGC UMR5095
Phone: 629669841
asen.daskalov@ibgc.cnrs.fr

Carol E Davis
UCR
Phone: (951) 827 3932
cdavis@ucr.edu

Charissa de Bekker
Pennsylvania State University
Phone: 814-321 2226
amd32@psu.edu

Ronnie de Jonge
VIB - PSB
Phone: 3293313537
ronnie.dejonge@psb.ugent.be

Ronald P de Vries
CBS-KNAW Fungal Biodiversity Centre
Phone: 31 302-122600
r.devries@cbs.knaw.nl

Ralph A Dean
North Carolina State Univ
Phone: (919) 513-0020
ralph_dean@ncsu.edu

Daniele Debieu
INRA
Phone: 33130814564
debieu@versailles.inra.fr

Robert Debuchy
Univ Paris-SUD
Phone: 33 1-69157012
robert.debuchy@igmors.u-psud.fr

Diego Delgado-Álvarez
CICESE
Phone: +52 646 1265163
ddelgado@cicese.edu.mx

Toni M Delorey
Broad Institute
Phone: 617-714-8225
delorey@broadinstitute.org

Steven H Denison
Eckerd College
Phone: (727) 864-8456
denisosh@eckerd.edu

Anne Dettmann
Molecular Plant Physiology
Phone: 0049-0761203266
adettma@gwdg.de

Sourabh Dhingra
Northern Illinois University
Phone: 815 753 1468
dhingra@niu.edu

Antonio Di Pietro
Univ de Cordoba
Phone: 34 957 2189 81
ge2dipia@uco.es

George Diallinas
University of Athens
Phone: 3.0210727465e+011
diallina@biol.uoa.gr

Marty Dickman
Plant Genomics and Biotechnolo
Phone: (979) 862-4788
mbdickman@tamu.edu

Axel CR Diernfellner
BZH
Phone: +49 6221 544778
axel.diernfellner@bzh.uni-heidelberg.de

Fred S Dietrich
Duke University
Phone: 919 684 2857
fred.dietrich@duke.edu

Daniela Dirschnabel
Ruhr-University Bochum
Phone: 4.923432277e+011
daniela.dirschnabel@rub.de

Peter Dodds
CSIRO
Phone: +61 2 62465039
peter.dodds@csiro.au

Gunther Doehlemann
Max Planck Institute
Phone: -7152
doehlemann@mpi-marburg.mpg.de

Bastian Doernte
University of Goettingen
Phone: 4.9551391409e+011
bdoernt@gwdg.de

Michael E Donaldson
Trent University
Phone: 705 748 1011
michaeldonald2@trentu.ca

Nicole M Donofrio
Univ Delaware
Phone: (302) 831-1372
ndonof@udel.edu

Tammy Doty
Novozymes, Inc.
Phone: 530 757-8100
tmmd@novozymes.com

Benjamin Doughan
University of Florida
Phone: 319-504-9168
bdoughan@ufl.edu

Damien Downes
Kansas State University
Phone: 785-532-1348
djdownes@ksu.edu

Kelly Drews
Virginia Tech
Phone: 7037281190
kcdrews@gmail.com

Bernard Dumas
UMR5546 CNRS Univ P Sabatier
Phone: 33 562193503
dumas@lrsv.ups-tlse.fr

Jay C Dunlap
Geisel Schl Medicine@Dartmouth
Phone: (603) 650-1108
jay.c.dunlap@dartmouth.edu

Sebastien Duplessis
INRA - Tree-Microbe Interactions
Phone: 33 383 394013
duplessi@nancy.inra.fr

Dannie Durand
Carnegie Mellon University
Phone: (412) 268-6036
durand@cmu.edu

Paul S Dyer
University of Nottingham
Phone: 44 115-9513203
paul.dyer@nottingham.ac.uk

Carla J Eaton
Massey University
Phone: 64 63569099
c.j.eaton@massey.ac.nz

Daniel J Ebbole
Texas A&M Univ
Phone: (979) 845-4831
d-ebbole@tamu.edu

Martin John Egan
Harvard Medical School
Phone: (617) 432-7177
martin_egan@hms.harvard.edu

Sophia K Ekengren
Stockholm Univ
Phone: 46 8 16 37 54
ekengren@botan.su.se

Candace E Elliott
University of Melbourne
Phone: (613)8344 5056
ce@unimelb.edu.au

Lynn Epstein
University of California
Phone: 530-754-7916
lepstein@ucdavis.edu

Nuria Escudero
University of Alicante
Phone: (+34)965903400 x 328
nuria.escudero@ua.es

Jose Espino
University of Muenster
Phone: 4.9251832481e+011
espino@uni-muenster.de

EDOUARD EVANGELISTI
INRA/CNRS/Université de Nice
Phone: 492386588
edouard.evangelisti@sophia.inra.fr

Jaime Eyzaguirre
Universidad Andres Bello
Phone: (562) 26618070
jeyzaguirre@unab.cl

David Ezra
ARO The Volcani Ctr
Phone: 972-3-9683555
dezra@volcani.agri.gov.il

Yohann Faivre Talmey
University Claude Bernard
Phone: 04 72 85 23 78
yohann.faivre@bayer.com

Rosa Fajardo
CICESE
Phone: 646 175 05 00
rfajardo@cicese.mx

Ahmad M Fakhoury
Southern Illinois Univ
Phone: (618) 453-1782
amfakhou@siu.edu

Weiguo FANG
Zhejiang University
Phone: 86-571-88206668
wfang1@zju.edu.cn

Yufeng Fang
Virginia Tech
Phone: 5408086725
yufengf1@vt.edu

Erzsébet Fekete
University of Debrecen
Phone: +3652512900 62488
kicsizsoka@yahoo.com

Michael Feldbrugge
Heinrich-Heine University
Phone: 49 211 15475
feldbrue@hhu.de

Daria Feldman
The Hebrew University of Jerusalem
Phone: 97289489315
daria.feldman@mail.huji.ac.il

Carl R Fellbaum
South Dakota State Univeristy
Phone: 6052167854
carl.fellbaum@sdstate.edu

Marianna Feretzaki
Duke Univ
Phone: (919) 684-2809
mf55@duke.edu

Caroline M Fernandes
Federal University
Phone: 55-21-25626573
cmota@biof.ufrj.br

Jessie Fernandez
University of Nebraska-Lincoln
Phone: (402) 613-6223
jfernandez99@huskers.unl.edu

LIST OF PARTICIPANTS

Melania Figueroa
Oregon State University
Phone: (541) 737-2234
figuerme@science.oregonstate.edu

Scott G Filler
Los Angeles Biomedical ResInst
Phone: (310) 222-3813
sfiller@ucla.edu

Sabine Fillinger
INRA
Phone: 33 1-3081 4565
sabine.fillinger@versailles.inra.fr

Amanda J Fischer
Novozymes Inc
Phone: (530) 757-8100
amfs@novozymes.com

Gregory J Fischer
Univ of Wisconsin-Madison
Phone: 715-574-8857
gfischer2@wisc.edu

Reinhard Fischer
Karlsruhe Inst. of Technology
Phone: 49 721-608-4630
reinhard.fischer@kit.edu

Joseph E Flaherty
Coker College
Phone: (843) 383-8079
jflaherty@coker.edu

Marko Flajsman
Univ of Ljubljana
Phone: 386 1 3203295
marko.flajsman@bf.uni-lj.sl

Damien Fleetwood
AgResearch
Phone: -7917
damien.fleetwood@agresearch.co.nz

Andre Fleissner
TU Braunschweig
Phone: 49 531-3915795
a.fleissner@tu-bs.de

Dimitrios Floudas
Clark University
Phone: (508) 847-5892
dfloudas@clarku.edu

Kathryn Ford
University of Bristol
Phone: 7738247075
Kathryn.Ford@bristol.ac.uk

Natasha (Tash) T Forester
AgResearch / University of Otago
Phone: 6463518315
natasha.forester@agresearch.co.nz

Jarrod R Fortwendel
University of South Alabama
Phone: 251-460-6681
jfortwendel@southalabama.edu

James M Fountaine
Scotland's Rural College
Phone: 4.4131535437e+011
james.fountaine@sruc.ac.uk

Gerda Fourie
FABI
Phone: +2712 420 3938
gerda1.fourie@fabi.up.ac.za

Elisabeth Fournier
INRA
Phone: 33 499624863
elisabeth.fournier@supagro.inra.fr

Thomas J Fowler
SIUE
Phone: (618) 650-5231
tfowler@siue.edu

William L Franck
NC State University
Phone: 919-513-0167
wlfranck@ncsu.edu

Rasmus J N Frandsen
Technical University of Denmark
Phone: 45 4525 2708
rasf@bio.dtu.dk

Stephen J Free
SUNY Univ, Buffalo
Phone: (716) 645-4935
free@acsu.buffalo.edu

Stanley Freeman
Volcani Ctr
Phone: 972 3-9683537
freeman@volcani.agri.gov.il

Daniela Freihorst
Friedrich-Schiller-Universität
Phone: +49 3641 949293
danielafreihorst@aol.com

Johannes Freitag
Philipps-Universität
Phone: 4.9642128271e+012
JohannesFreitag@gmx.net

Michael Freitag
Oregon State Univ
Phone: (541) 737-4845
freitagm@onid.orst.edu

Fernanda Z Freitas
IQ-UNESP
Phone: 55-16-33019804
fernanda.zanolli@gmail.com

Steven Friedman
Oregon State University
Phone: (410) 967-0974
stevenfriedman8@gmail.com

Timothy L Friesen
USDA-ARS
Phone: 701-239-1337
timothy.friesen@ars.usda.gov

Ci Fu
University at Buffalo
Phone: (716)645-4936
cifu@buffalo.edu

Makoto Fujimura
Toyo Univ
Phone: 81 276-82-9216
mfujimura@toyo.jp

Fumi Fukada
Kyoto Prefectural University
Phone: 81-75-703-5613
ddswb383@yahoo.co.jp

Kevin K Fuller
Geisel Sch Medicine at Dartmouth
Phone: (603) 650-1120
kevin.fuller@dartmouth.edu

James Galagan
Boston University
Phone: 617 875 9874
jgalag@bu.edu

Jonathan M Galazka
Oregon State University
Phone: 541-737-4399
galazkaj@onid.orst.edu

JORGE GARCÍA-MARTÍNEZ
University of Seville
Phone: +34 954 555 948
jorgegarcia@us.es

Donald M Gardiner
CSIRO
Phone: 61-7-32142370
donald.gardiner@csiro.au

Andrea Gargas
Symbiology LLC
Phone: (608) 827-5164
andreagargas@symbiology.com

Victoriano Garre
University of Murcia
Phone: 34 868887148
vgarre@um.es

Amruta Garud
UC, Riverside
Phone: 858-231-7773
amruta.garud@email.ucr.edu

Audrey P Gasch
Univ Wisconsin, Madison
Phone: (608) 265-0859
agasch@wisc.edu

Elodie Gaulin
LRSV UMR5546 CNRS/UPS
Phone: +33 534323803
gaulin@lrsv.ups-tlse.fr

Gregory M. Gauthier
University of Wisconsin
Phone: (608) 262-7703
gmg@medicine.wisc.edu

Anupama Ghosh
MPI Marburg
Phone: 0049-6421-178571
anupama.ghosh@mpi-marburg.mpg.de

Arit Ghosh
University of California, Riverside
Phone: 951-827-3190
aghos003@ucr.edu

Sarah A Gilmore
UCSF
Phone: 4155024810
sarah.gilmore@ucsf.edu

MARTHA C GIRALDO
KANSAS STATE UNIVERSITY
Phone: (785) 532-2337
mgiraldo@ksu.edu

Tatiana Giraud
CNRS- Univ Paris 11
Phone: 33 1 6915-5669
tatiana.giraud@u-psud.fr

Pierre Gladieux
UC Berkeley
Phone: 510-642-8441
pgladieux@berkeley.edu

Louise Glass
Univ California
Phone: (510) 643-2399
lglass@berkeley.edu

Scott E Gold
USDA-ARS
Phone: (706) 542-1259
sgold@uga.edu

Katsuya Gomi
Tohoku Univ
Phone: 81 22-717-8901
gomi@biochem.tohoku.ac.jp

Rodrigo D Gonçalves
instituto de Química, UNESP
Phone: 55 (16) 82007360
rdgoncalves@gmail.com

Stephen B Goodwin
USDA-ARS/Purdue Univ
Phone: (765) 494-4635
sgoodwin@purdue.edu

Mathieu Gourgues
Université de Nice
Phone: 33492386588
mathieu.gourgues@sophia.inra.fr

Francine Govers
Wageningen Univ
Phone: 31 317-483-138
francine.govers@wur.nl

Neil A R Gow
Univ Aberdeen
Phone: 44 1224-437483
n.gow@abdn.ac.uk

Jonathan Grandaubert
INRA
Phone: 33130814569
jonathan.grandaubert@versailles.inra.fr

Fabrice N Gravelat
McGill University
Phone: 1 (514) 398-4434
fabrice.gravelat@mcgill.ca

Dave Greenshields
Novozymes BioAg Ltd
Phone: 3066578269
dvgs@novozymes.com

Laura J Grenville-Briggs
Royal Institute of Technology
Phone: +46 (0)5537 8890
lauragb@kth.se

LIST OF PARTICIPANTS

Igor V Grigoriev
Joint Genome Inst
Phone: (925) 296-5860
ivgrigoriev@lbl.gov

Pierre Grognet
Université Paris Sud
Phone: 33169154657
pierre.grognet@igmors.u-psud.fr

Guido Grossmann
University of Heidelberg
Phone: -551832
grossmann@stanford.edu

Alexey A. Grum-Grzhimaylo
Wageningen University
Phone: 31684940430
alexey.grumgrzhimaylo@wur.nl

Elisabeth Grund
Bayer CropScience
Phone: 33649116097
elisabeth.grund@bayer.com

Andrii Gryganskyi
Duke University
Phone: 9196607363
apg10@duke.edu

Fabio Gsaller
Medical Univ of Innsbruck
Phone: 0043 650 6617957
Fabio.Gsaller@i-med.ac.at

Matthias Gube
FSU Jena
Phone: 4.936419494e+011
matthias.gube@uni-jena.de

JANBON Guilhem
Institut Pasteur
Phone: 33 1 45688356
janbon@pasteur.fr

Thomas Guillemette
University of Angers
Phone: 33241735260
thomas.guillemette@univ-angers.fr

Amy M Gundersen
Novozymes, Inc.
Phone: 559-706-0062
aygm@novozymes.com

Yogesh K Gupta
University of Exeter
Phone: -7404494002
ykg201@exeter.ac.uk

sarah j gurr
Univ oxford
Phone: 4.4186527581e+011
sarah.gurr@plants.ox.ac.uk

Paulina Guzman-Guzman
University of Guanajuato
Phone: 473 1090556
paulina.gzmn2@gmail.com

Rachana Gyawali
Texas A&M University
Phone: (979) 845-7259
rgyawali@bio.tamu.edu

Ulrich Güldener
Helmholtz-Zentrum München
Phone: +49 89 3187 3582
u.gueldener@helmholtz-muenchen.de

Thomas Haarmann
AB Enzymes GmbH
Phone: 4.9615136804e+012
thomas.haarmann@abenzymes.com

Hubertus Haas
Innsbruck Medical University
Phone: -11363969
hubertus.haas@i-med.ac.at

Yitzhak Hadar
Hebrew Unversity
Phone: 972-8-9489935
hadar@agri.huji.ac.il

Stefan A Haefner
BASF SE
Phone: +49 621 60-42902
stefan.haefner@basf.com

Daisuke Hagiwara
Chiba university
Phone: -2983
dhagi@chiba-u.jp

Charles R Hall
Dupont Industrial Biosciences
Phone: (510) 350-7426
charles.hall@dupont.com

Heather E Hallen-Adams
University of Nebraska-Lincoln
Phone: (402) 472-2825
hhallen-adams2@unl.edu

Richard C Hamelin
University of British Columbia
Phone: 604-827-4441
richard.hamelin@ubc.ca

Kim E Hammond-Kosack
Rothamsted Research
Phone: 44-1582-763133
kim.hammond-kosack@rothamsted.ac.uk

Jae-Gu Han
Rural Development Administration
Phone: -4631180
jaeguhan@gmail.com

Kap-Hoon Han
Woosuk Univ
Phone: 82 63-290-1427
khhan@woosuk.ac.kr

Christopher T Hann-Soden
UC Berkeley
Phone: 9258722716
channsoden@berkeley.edu

Wilhelm Hansberg
Inst. Fisiología Celular/UNAM
Phone: 5255 5622 5655
whansberg@ifc.unam.mx

Omar Harb
Univ of Pennsylvania
Phone: 215-746-7019
oharb@pcbi.upenn.edu

Jessica Hargarten
University of Nebraska- Lincoln
Phone: 925-300-7007
jessica.c.teixeira@gmail.com

Steven D Harris
Univ Nebraska
Phone: (402) 472-2938
sharris2@unl.edu

Thomas Hartmann
Chinese Academy of Sciences
Phone: 0086 1860 237 384
hartmann.tom@gmail.com

Sahar Hasim
Univesity of Nebraska Lincoln
Phone: 402-2022326
saharm@huskers.unl.edu

Shin Hatakeyama
Saitama Univ
Phone: 81-48-858-3414
shinh@mail.saitama-u.ac.jp

Virginia E Havel
University at Buffalo
Phone: 7168292091
vehavel@buffalo.edu

Patrick M Hayes
University of Bristol
Phone: 44117 928 7475
patrick.hayes@bristol.ac.uk

Kim Hee-Kyoung
Soonchunhyang University
Phone: -1777
beargn@naver.com

Yvonne Heilig
Molecular Plant Physiology
Phone: 4.9761203267e+011
yheilig@gwdg.de

Kai Heimel
Georg-August-University
Phone: 49551-393815
kheimel@gwdg.de

Joseph Heitman
Duke Univ
Phone: 919-684-2824
heitm001@duke.edu

Jens Heller
UC-Berkeley
Phone: 510-501-3152
jens.heller@berkeley.edu

Ginnie K Hench
UNC Chapel Hill
Phone: (919) 923-2309
ghench@med.unc.edu

Catarina Henke
Friedrich-Schiller Univ - MPI
Phone: 49 03461 949299
chenke@ice.mpg.de

Bernard Henrissat
CNRS
Phone: 33 49182 55 87
Bernard.Henrissat@afmb.univ-mrs.fr

Yainitza Hernandez-Rodriguez
Univ Georgia
Phone: (706) 542-6026
yrodriguez@plantbio.uga.edu

Andreas Herr
Karlsruhe Inst of Technolog
Phone: 4.9721608446e+012
andreas.herr@kit.edu

Alfredo H Herrera-Estrella
CINVESTAV-IPN, Irapuato
Phone: 52-462-1663041
aherrera@langebio.cinvestav.mx

Andrea Herrmann
WWU Muenster
Phone: 4.9251832499e+011
andrea.herrmann@uni-muenster.de

Christian Hertweck
HKI
Phone: +49 3641 5321100
christian.hertweck@hki-jena.de

Britta Herzog
Georg-August-University
Phone: 4.9551391967e+011
bherzog@gwdg.de

Jaqueline Hess
Harvard University
Phone: 617-496-9741
jhess@fas.harvard.edu

Ana G Hesselbart Marquez
Carlsberg Laboratory
Phone: 4533275235
yonosoyana@gmail.com

David S Hibbett
Clark University
Phone: 508-793-7332
dhibbett@clarku.edu

Yujiro Higuchi
University of Exeter
Phone: +44 (0) 1392 269178
Y.Higuchi@exeter.ac.uk

Kristiina Hilden
Univ Helsinki
Phone: 35891911
kristiina.s.hilden@helsinki.fi

Terry W Hill
Rhodes College
Phone: 901-843-3559
hill@rhodes.edu

Robert Louis Hirsch
University of Arkansas
Phone: 479-575-4952
rhirsch@uark.edu

Andrea Hlubek
BASF
Phone: +49 621 60-59338
andrea.a.hlubek@basf.com

Dirk Hoffmeister
Friedrich-Schiller-University
Phone: 01149-3641-949850
dirk.hoffmeister@hki-jena.de

LIST OF PARTICIPANTS

Linda Holland
University College Dublin
Phone: 3.5387664498e+011
linda.holland@ucd.ie

William K Holloman
Cornell Univ Weill Med Col
Phone: (212) 746-6510
wkhollo@med.cornell.edu

Shinji Honda
University of Fukui
Phone: -9456
SHINJI@u-fukui.ac.jp

Christian Hong
University of Cincinnati
Phone: 5135583289
christian.hong@uc.edu

Sung-Yong Hong
Michigan State University
Phone: 517-353-2270
hongsun7@msu.edu

Chiaki Hori
University of Tokyo
Phone: -11020
chori718@jcom.home.ne.jp

Benjamin A Horwitz
Technion-ITT
Phone: 972 4 8293976
horwitz@tx.technion.ac.il

Sara Hosseini
SLU
Phone: 0046-18-672379
sara.hosseini@slu.se

Petra Houterman
University of Amsterdam
Phone: +31 20 525 7706
P.M.Houterman@uva.nl

Barbara J Howlett
Univ Melbourne
Phone: 61 3-8344-5062
bhowlett@unimelb.edu.au

Yen-Ping Hsueh
California Inst of Technology
Phone: (626) 395-2669
yph@caltech.edu

Yuhong Huang
Aalborg University
Phone: +45 99402596
huyu@bio.aau.dk

Richard Hung
Rutgers, The State University of New Jersey
Phone: (732) 850-6220
Richard.Hung@rutgers.edu

Cameron Hunter
Kansas State University
Phone: 913-907-8289
cameron.hunter15@gmail.com

Jae-Seoun Hur
Sunchon National University
Phone: -4112
jshur1@sunchon.ac.kr

David Huskey
University of Arizona
Phone: 520-621-9390
dahuskey@email.arizona.edu

Michael J Hynes
Univ Melbourne
Phone: 61 03-83446239
mjhynes@unimelb.edu.au

Nils OS Högberg
BioCenter, SLU
Phone: 4618671875
nils.hogberg@slu.se

Akihiko Ichiishi
Toyo University
Phone: 81 276-82-9203
akihiko@toyo.jp

Alexander Idnurm
Univ Missouri-Kansas City
Phone: (816) 235-2265
idnurma@umkc.edu

Kiyohiko Igarashi
University of Tokyo
Phone: 81-3-5841-5258
aquarius@mail.ecc.u-tokyo.ac.jp

Patrik Inderbitzin
UC Davis
Phone: 916 668 9078
patrik.inderbitzin@gmail.com

Diane O Inglis
Stanford University
Phone: (415) 713-1408
dianeoinglis@gmail.com

Vong shian Simon Ip Cho
University of Copenhagen
Phone: 4535332627
sich@life.ku.dk

Akira Ishii
Tokyo Univ. of Science
Phone: -16194
j6412701@ed.noda.tus.ac.jp

Kazi Tariqul Islam
Southern Illinois University
Phone: 6182033089
tariq@siu.edu

Christa Ivanova
Technical University Vienna
Phone: +43 6801127145
christa.ivanova@tuwien.ac.at

Kazuhiro Iwashita
National Research Institute of Brewing
Phone: -2688
iwashitact@nrib.go.jp

Sultana N Jahan
Sveriges lantbruksuniversitet
Phone: +46 18-673238
sultana.jahan@slu.se

Kwang-Yeop Jahng
Chonbuk Natl Univ Col Nat Sci
Phone: 82 63-270-3358
goodear@chonbuk.ac.kr

Jernej Jakse
Univ. of Ljubljana
Phone: 386 1 3203280
jernej.jakse@bf.uni-lj.sl

Branka Javornik
Biotechnical Faculty
Phone: +386 1 3203260
branka.javornik@bf.uni-lj.si

Gregory Jedd
Phone: 65 93380462
gregory@tll.org.sg

Junhyun Jeon
Seoul National University
Phone: 82-2-880-4684
plantdr1@snu.ac.kr

Qiming Jin
Novozymes Inc
Phone: (530) 757-0827
qmji@novozymes.com

Seongmi Jo
Soonchunhyang Univ.
Phone: -1777
kinosjo@gmail.com

Hanna Johannesson
Uppsala Univ
Phone: 46 18 4716662
hanna.johannesson@ebc.uu.se

Annette H Johansen
Novozymes A/S
Phone: (0045)44460736
ahjo@novozymes.com

Tomas Johansson
Lund University
Phone: 46 46-222-4549
tomas.johansson@biol.lu.se

Anna Johns
Univ. of Manchester
Phone: 447 7205 62237
anna.johns@postgrad.manchester.ac.uk

Derek Johnson
Oregon State University
Phone: 541-737-5302
johnsde4@science.oregonstate.edu

Linda J Johnson
AgResearch Limited
Phone: 6463518189
linda.johnson@agresearch.co.nz

Richard D Johnson
Agresearch Grasslands
Phone: +64 6 3518090
richard.johnson@agresearch.co.nz

David L Joly
Agriculutre and Agri-Food Canada
Phone: (250) 404-3318
David.Joly@agr.gc.ca

Wilfried Jonkers
UC Berkeley
Phone: 510-643-2546
wjonkers@berkeley.edu

Rob Joosten
Dyadic Nederland BV
Phone: +31 317 465456
rjoosten@dyadic.nl

Howard S Judelson
Univ California
Phone: (951) 827-4199
howard.judelson@ucr.edu

Boknam Jung
Dong-A University
Phone: 82-51-200-5670
wjdqhrska89@naver.com

Elke-Martina Jung
Friedrich Schiller University
Phone: 1.1493641949e+013
elke-martina.jung@uni-jena.de

KWANG-WOO JUNG
Yonsei University
Phone: 82-70-7578-1596
jkwoo1983@hanmail.net

Praveen R Juvvadi
Duke University Medical Center
Phone: 919-681-2613
praveen.juvvadi@duke.edu

Bastian Jöhnk
Georg August University
Phone: +49 551 393821
bjoehnk@gwdg.de

Maciej Kaczmarek
Scotland's Rural College
Phone: 7706957947
maciej.kaczmarek@sruc.ac.uk

Joerg T Kaemper
KIT
Phone: 49 721 609 45670
joerg.kaemper@kit.edu

Meenakshi Kagda
UC, Riverside
Phone: 951-756-6400
mkagd001@ucr.edu

Regine Kahmann
MPI Terrestrial Microbiology
Phone: 49 6421-178501
kahmann@mpi-marburg.mpg.de

Shiv D Kale
Virginia Tech.
Phone: (540)-231-3784
sdkale@vt.edu

Vincent Kam
Novozymes, Inc.
Phone: 530-757-8100 x 4624
vck@novozymes.com

Hisae Kamakura
Tokyo Univ. of Science
Phone: -16194
rheingold@i.softbank.jp

Takashi Kamakura
Tokyo Univ. of Science
Phone: -16194
kamakura@rs.noda.tus.ac.jp

LIST OF PARTICIPANTS

Masayuki Kamei
Toyo University
Phone: -9493
dx110002x@toyo.jp

Susan G W Kaminskyj
University of Saskatchewan
Phone: +1 306 966 4422
susan.kaminskyj@usask.ca

Zhian N. Kamvar
Oregon State University
Phone: (541) 738-4074
kamvarz@science.oregonstate.edu

Seogchan Kang
Pennsylvania State Univ
Phone: (814) 863-3846
sxk55@psu.edu

Levente Karaffa
University of Debrecen
Phone: +3652512900 62488
karaffa.levente@science.unideb.hu

Margaret E Katz
Univ New England
Phone: 61 2-6773-3016
mkatz@une.edu.au

Jan Naseer Kaur
University at Buffalo
Phone: 716-829-2091
jannasee@buffalo.edu

Moriyuki Kawauchi
Hiroshima University
Phone: -1245
m-kawauchi@nrib.go.jp

Nancy P Keller
Univ Wisconsin, Madison
Phone: (608) 262-9795
npkeller@wisc.edu

Ronny Kellner
Max Planck Institute Marburg
Phone: 49 642 1178 632
ronny.kellner@mpi-marburg.mpg.de

Frank Kempken
Christian-Albrechts University
Phone: 49 431-880-4274
fkempken@bot.uni-kiel.de

John C Kennell
St Louis Univ
Phone: (314) 977-3905
kennellj@slu.edu

Nemat O Keyhani
Univ Florida
Phone: (352) 392-2488
keyhani@ufl.edu

Dae-Hyuk Kim
Chonbuk National Univ
Phone: 82 63 270 3440
dhkim@chonbuk.ac.kr

Hye-Seon Kim
Univ of Delaware
Phone: 302-831-3429
hyeseon@udel.edu

Jung-Eun Kim
Penn State University
Phone: 814-863-2074
juk30@psu.edu

Jung-Mi Kim
Wonkwang University
Phone: 82-63-850-6676
micro@wku.ac.kr

Seongbeom Kim
Seoul National University
Phone: -5434
ssungbum@snu.ac.kr

Corby Kistler
Cereal Disease Laboratory
Phone: (612) 625-9774
hckist@umn.edu

Sylvia Klaubauf
CBS-KNAW Fungal Biodiversity Centre
Phone: 31302122600
s.klaubauf@cbs.knaw.nl

Stefan Klinter
Royal Institute of Technology
Phone: 46735570644
klinter@kth.se

Andrew D. Klocko
University of Oregon
Phone: 541-346-5197
klocko@uoregon.edu

Steve J Klosterman
USDA-ARS
Phone: (831) 755-2845
Steve.Klosterman@ars.usda.gov

Olaf Kniemeyer
Leibniz Institute for Natural Product Research and Infection Biology (HKI)
Phone: (0049) 3641-5321071
olaf.kniemeyer@hki-jena.de

Tetsuo Kobayashi
Nagoya University
Phone: 81527894085
koba@agr.nagoya-u.ac.jp

Sayo Kodama
Kyoto Prefectural University
Phone: 81-75-703-5613
ze14skodama@gmail.com

Annegret Kohler
INRA Centre de Nancy
Phone: +33 383394072
kohler@nancy.inra.fr

Linda M Kohn
Univ Toronto Mississauga
Phone: 905-277-1321
linda.kohn@utoronto.ca

Hideaki Koike
AIST
Phone: 81 29-861-6679
hi-koike@aist.go.jp

Kaihei Kojima
Novozymes Japan Ltd.
Phone: -7028
kka@novozymes.com

Anja Kombrink
Wageningen University
Phone: 0031-317485325
anja.kombrink@wur.nl

Michael Koonce
National Science Foundation
Phone: 702-292-7144
mkoonce@nsf.gov

Branka Korosec
National Institute of Chemistry
Phone: 38614760262
branka.korosec@ki.si

Erika Kothe
Friedrich-Schiller University
Phone: 49 3641-949291
erika.kothe@uni-jena.de

Lukasz Kozubowski
Duke Universtiy Medical Center
Phone: (919) 308-5974
lukasz.kozubowski@duke.edu

Nada Krasevec
National Institute of Chemistry
Phone: +386(1)4760-262
nada.krasevec@ki.si

Katrin Krause
Friedrich Schiller University
Phone: +49 (0)3641-949399
Katrin.Krause@uni-jena.de

Pauline Krijgsheld
Utrecht University
Phone: 31302533041
p.krijgsheld@uu.nl

Brent A Kronmiller
Oregon State University
Phone: 510-825-8153
bkronmiller@gmail.com

James W Kronstad
Univ British Columbia
Phone: (604) 822-4732
kronstad@interchange.ubc.ca

Yasuyuki Kubo
Kyoto Prefectural University
Phone: 81 75-703-5613
y_kubo@kpu.ac.jp

Ulrich Kueck
Ruhr Univ Bochum
Phone: 49 234-3226212
ulrich.kueck@rub.de

Ursula Kues
Georg-August Univ
Phone: 49 551-397024
ukuees@gwdg.de

Gretchen Kuldau
Penn State
Phone: 814-863-7232
kuldau@psu.edu

Abhishek Kumar
Institute of Botany, CAU Kiel
Phone: 4.9179759067e+011
akumar@bot.uni-kiel.de

Emi Kunitake
Osaka Prefecture University
Phone: 81-72-254-9466
kunitake@biochem.osakafu-u.ac.jp

Markus Kunzler
ETH Zurich
Phone: 41 446324925
markus.kuenzler@micro.biol.ethz.ch

Alan Kuo
DOE Joint Genome Inst
Phone: 925-899-1364
akuo@lbl.gov

Kiminori Kurashima
Saitama Univercity
Phone: 81-48-858-3414
s09db004@mail.saitama-u.ac.jp

Jaana T Kuuskeri
University of Helsinki
Phone: 3.5891915928e+011
jaana.kuuskeri@helsinki.fi

Hoi S Kwan
Chinese Univ, Hong Kong
Phone: 85239436251
hoishankwan@cuhk.edu.hk

Gerald Lackner
University Jena
Phone: +49 17623132017
gerald.lackner@hki-jena.de

Ljerka Lah
National Inst. of Chemistry
Phone: 38641384557
Iljerka.lah@ki.si

Herve Lalucque
Université Paris Sud
Phone: 33 1-69154668
herve.lalucque@igmors.u-psud.fr

Scott Lambie
University of British Columbia
Phone: 604 822 2217
scott.lambie@gmail.com

Elza A. S. Lang
University of Sao Paulo
Phone: 5516 3602-3078
elzalang@usp.br

Thorsten Langner
Heinrich-Heine University
Phone: 0049 (0)211 81 11529
thorsten.langner@hhu.de

Nicolas NL LAPALU
INRA- URGI
Phone: 33130833810
nicolas.lapalu@versailles.inra.fr

Luis F Larrondo
Pontificia Universidad Católica de Chile
Phone: (56)-2-23541916
llarrondo@bio.puc.cl

Jennifer R Larson
Ohio State Univ
Phone: (614) 247-6873
larson.315@osu.edu

LIST OF PARTICIPANTS

Olga Lastovetsky
Cornell University
Phone: 607-220-7351
ol57@cornell.edu

Linda Lauinger
BZH
Phone: +49 6221 544345
linda.lauinger@bzh.uni-heidelberg.de

Michelle Leach
University of Toronto
Phone: (647) 405-4437
michelle.leach@abdn.ac.uk

Marc-Henri Lebrun
INRA
Phone: (33) 130814551
marc-henri.lebrun@versailles.inra.fr

In H Lee
Kookmin Univ
Phone: 82 2-910-4771
leei@kookmin.ac.kr

JUNGKWAN LEE
Dong-A University
Phone: 82-51-200-7554
jungle@dau.ac.kr

Mi Kyung Lee
University of Wisconsin Madison
Phone: 6082636830
mlee234@wisc.edu

SooChan Lee
Duke University Medical Center
Phone: 919-684-3036
soochan.lee@duke.edu

Yong-Hwan Lee
Seoul National Univ
Phone: 82 2-880-4674
yonglee@snu.ac.kr

Yoon Ji Lee
Seoul National Univ
Phone: -5481
damnkool@snu.ac.kr

François Lefebvre
Universite Laval
Phone: (418) 977-0963
francois.lefebvre.3@ulaval.ca

Ronny Lehneck
Georg-August-University
Phone: 49551393579
rlehnec@gwdg.de

Taija Leinonen
Roal Oy
Phone: 3.5892904216e+011
taija.leinonen@roal.fi

Yueqiang Leng
North Dakota State University
Phone: 6122290411
yueqiang.leng@ndsu.edu

Klaus B Lengeler
Carlsberg Laboratory
Phone: +45 3327 5236
KlausB.Lengeler@carlsberglab.dk

Sophie Lev
University of Sydney
Phone: 61-2-98457046
levsophie@gmail.com

Stuart Levitz
University of Massachusetts
Phone: 508-856-1525
stuart.levitz@umassmed.edu

Zachary A Lewis
University of Georgia
Phone: (541) 346-5197
zlewis@uga.edu

Guotian Li
Phone: 765-237-2617
leeguotian@163.com

Simeng Li
Beijing Forestry University
Phone: +86 18611426618
lisimeng00@gmail.com

Xiangqian Li
Monsanto
Phone: 63017
xiangqian.li@monsanto.com

Xiaofei Liang
Phone: (352)-328-9853
xiaofeiliang@ufl.edu

Hui-Ling Liao
Duke Univ
Phone: 9196607362
sunny.liao@duke.edu

Edward Yi-Hong Liaw
Univ of California, Riverside
Phone: (949)682-8289
edward.liaw@ucr.edu

Alex Lichius
Vienna Univ of Technology
Phone: +43(1)58801-166524
alexlichius@gmail.com

Fang Yun Lim
University of Wisconsin-Madison
Phone: 608-262-1958
fylim@wisc.edu

Ching-Hsuan Lin
National Taiwan University
Phone: 886-2-33664449
chinghsuanlin@ntu.edu.tw

Xiaorong Lin
Texas A&M University
Phone: (979) 845-7274
xlin@bio.tamu.edu

Björn D Lindahl
SLU
Phone: +46 18 672725
Bjorn.Lindahl@slu.se

Haoping Liu
Univ California Col Medicine
Phone: (949) 824-1137
h4liu@uci.edu

Huiquan Liu
Northwest A&F University
Phone: 86-29-87081270
liuhuiquan@nwsuaf.edu.cn

Yi Liu
UT Southwestern Medical Ctr
Phone: (214) 645-6033
yi.liu@utsouthwestern.edu

Zhaohui Liu
North Dakota State University
Phone: (701) 231-7454
zhh.liu@ndsu.edu

Lars Lobach
School of Biological Sciences
Phone: 4.475346927e+011
lars.loebach@abdn.ac.uk

Jennifer K Lodge
Washington Univ Sch Med
Phone: 314-747-0515
lodgejk@wustl.edu

Luis V Lopez-Llorca
Univeristy Alicante
Phone: 34-965903400
lv.lopez@ua.es

Federico Lopez-Moya
University of Alicante
Phone: 0034965903400 x3280
federicolopezmoya@gmail.com

Jennifer Lorang
Oregon State University
Phone: 541-737-5282
lorangj@science.oregonstate.edu

Jennifer J Loros
Geisel Sch Medicine@Dartmouth
Phone: (603) 650-1154
jennifer.loros@dartmouth.edu

Edward J Louis
Univ Nottingham
Phone: 44 115-823-0375
ed.louis@nottingham.ac.uk

Rohan G T Lowe
University of Melbourne
Phone: 61383445056
rohan.lowe@unimelb.edu.au

Ling Lu
Nanjing Normal Univeristy
Phone: 86-25-85891791
linglu6465@hotmail.com

Taina K Lundell
University of Helsinki
Phone: +358 9 191 59316
taina.lundell@helsinki.fi

Hong Luo
Michigan State Univ
Phone: (517) 353-4886
hongluo@msu.edu

Liang Ma
SIBS,CAS
Phone: 86-21-54924049
liangm2008@yahoo.com

Darel Anne Macdonald
University of Manchester
Phone: 4.4759566856e+011
darel.macdonald@postgrad.manchester.ac.uk

Juliane Macheleidt
Hans-Knoell-Institute
Phone: 4.9364153213e+012
Juliane.Macheleidt@hki-jena.de

Masayuki Machida
Natl Inst Advanced Sci Tech
Phone: 81 298-61-9447
m.machida@aist.go.jp

Soumya Madhavan
Friedrich Schiller University
Phone: 4.936419494e+011
soumya.madhavan@uni-jena.de

Lori A Maggio-Hall
EI Dupont de Nemours & Co
Phone: (302) 695-1480
lori.a.maggio-hall@usa.dupont.com

Uma Maheswari
EMBL-EBI
Phone: 4.4122349468e+011
uma@ebi.ac.uk

Alejandra Mandel
University of Arizona
Phone: (520) 621-9419
mandel@ag.arizona.edu

Viola A Manning
Oregon State Univ
Phone: (541) 737-2234
manniv@science.oregonstate.edu

Elisabetta Marchegiani
INRA
Phone: 033-130814560
elisabetta.marchegiani@versailles.inra.fr

Mark R Marten
Univ Maryland,Baltimore County
Phone: (410) 455-3439
marten@umbc.edu

Francis M Martin
INRA
Phone: 33 383-394080
fmartin@nancy.inra.fr

Leonora Martinez
CICESE
Phone: 175 0500
lotoleias@gmail.com

Ana Lilia Martinez-Rocha
University of Hamburg
Phone: +49 40 428 16 309
analiliamartinezrocha@googlemail.com

Nilce M Martinez-Rossi
Sao Paulo University
Phone: 55 16-36023150
nmmrossi@usp.br

Emma Master
University of Toronto
Phone: 416-946-7861
emma.master@utoronto.ca

Elisabeth Maurer
Medical University Innsbruck
Phone: -80204
elisabeth.maurer@i-med.ac.at

LIST OF PARTICIPANTS

Georgiana May
Univ Minnesota
Phone: (612) 624-6737
gmay@umn.edu

Kevin McCluskey
Univ Missouri, Kansas City
Phone: (816) 235-6484
mccluskeyk@umkc.edu

Bruce A McDonald
ETH-Zentrum, LFW B16
Phone: 41 44-632-3848
bruce.mcdonald@usys.ethz.ch

Tami R McDonald
University of Minnesota
Phone: (612) 625-4975
mcdo0264@umn.edu

Matt Mead
University of Wisconsin-Madison
Phone: 608-265-5689
mmead@wisc.edu

Pankaj Mehrotra
Aberdeen Fungal Group
Phone: 0044-01224 4374
p.mehrotra@abdn.ac.uk

Harold Meijer
Wageningen University
Phone: 31 317-483138
harold.meijer@wur.nl

Hugo Melida
Royal Inst of Technology (KTH)
Phone: -13869
hmelida@kth.se

Artemio Mendoza
Lincoln Univ
Phone: +64 3321 8472
artemio.mendoza@lincoln.ac.nz

El Ghalid Mennat
Universidad de Cordoba
Phone: 0034 682280250
m.elghalid@gmail.com

Martha W Merrow
Ludwig-Maximilians-Universität-München
Phone: 4.9171381266e+011
merrow@lmu.de

Alexander V Michkov
University of California, Riverside
Phone: (951)8273190
alexander.michkov@email.ucr.edu

Santiago Xavier Mideros
Cornell University
Phone: (607)339-8711
sxm2@cornell.edu

Kyunghun Min
Seoul National Univ
Phone: 82 2-8804681
mymin117@snu.ac.kr

Anna Minz-Dub
Tel-Aviv University
Phone: 9.725447883e+011
annamin1@post.tau.ac.il

Alejandro Miralles-Duran
Facultad de Biologia
Phone: +34 954556473
amiralles@us.es

Amir Mirzadi Gohari
Plant Research International (PR
Phone: 686323223
amir.mirzadigohari@wur.nl

Amanda L Misener Bloom
SUNY University at Buffalo
Phone: 716-829-2091
amisener@buffalo.edu

Aaron P Mitchell
Carnegie Mellon Univ
Phone: (412) 268-5844
apm1@andrew.cmu.edu

Yasumasa Miyazaki
FFPRI
Phone: -9056
ymiyazak@ffpri.affrc.go.jp

Caroline Moffat
Curtin University
Phone: 61892669186
Caroline.Moffat@curtin.edu.au

Venkatesh Moktali
Penn State Univ
Phone: (814) 321-5762
vpm104@psu.edu

Michelle C Momany
Univ Georgia
Phone: (706) 542-2014
momany@plantbio.uga.edu

Stephen J Mondo
Cornell University
Phone: (607) 342-8087
sjm284@cornell.edu

Valdirene Monteiro
Universidade Estadual de Goiás
Phone: +55(62)33281160
valdirene.neves@ueg.br

Melanie Morel
INRA
Phone: 999 0383684228
Melanie.Morel@scbiol.uhp-nancy.fr

Marino Moretti
MPI for Terrestrial Microbiology
Phone: 49 0 6421 178 521
marino.moretti@mpi-marburg.mpg.de

Paul F Morris
Bowling Green State Univ
Phone: (419) 372-0481
pmorris@bgsu.edu

Uffe H Mortensen
DTU Systems Biology
Phone: 45 4525 2701
um@bio.dtu.dk

Rosa R Mourino-Perez
CICESE
Phone: 52 646-1750500
rmourino@cicese.mx

Donna L Moyer
Novozymes Inc
Phone: (530) 757-8115
dlm@novozymes.com

Andre Mueller
MPI for Terrestrial Microbiology
Phone: 49 642 1178 612
andre.mueller@mpi-marburg.mpg.de

Alija Bajro Mujic
Oregon State University
Phone: (541) 737-5302
mujica@science.oregonstate.edu

Alberto Muñoz
University of Edinburgh
Phone: (+44)-(0)1316513337
alberto.munoz@ed.ac.uk

Hajime Muraguchi
Akita Prefectural Univ
Phone: 81 18-872-1580
muraguchi@akita-pu.ac.jp

Gloria Muzzi Erichsen
Novozymes Inc.
Phone: 15307578100
GMEr@novozymes.com

Miia R Mäkelä
Univ Helsinki
Phone: 3.5891915931e+011
miia.r.makela@helsinki.fi

Maryam Nadimi
Univeristy of Montreal
Phone: (514)746-2624
maryam.nadimi@umontreal.ca

Vikram Naik
MPI for Terrestrial Microbiology
Phone: 49 6421 178 522
naik@mpi-marburg.mpg.de

Mizue Naito
Cornell University
Phone: 607-280-3774
mn389@cornell.edu

Masaya Nakamura
FFPRI
Phone: -9059
nmasaya@ffpri.affrc.go.jp

Mayumi Nakayama
Tohoku University
Phone: -9437
mayumi-n@niche.tohoku.ac.jp

Takehito Nakazawa
University of Shizuoka
Phone: 81 54 264-5662
gp1595@u-shizuoka-ken.ac.jp

Andre Nantel
National Research Council
Phone: 1 (514) 496-6370
Andre.Nantel@nrc-cnrc.gc.ca

Naweed I Naqvi
Temasek Life Sciences Laboratory
Phone: 65-68727493
naweed@tll.org.sg

Gesabel Y Navarro Velasco
Universidad de Cordoba
Phone: 0034 957212421
z72naveg@uco.es

Attila Nemeth
Codexis
Phone: -6921
attila.nemeth@codexis.com

Helena KM Nevalainen
Macquarie University
Phone: 61403162038
helena.nevalainen@mq.edu.au

Connie B Nichols
Duke Medical Ctr
Phone: (919) 684-5054
connie.nichols@duke.edu

Francisco E Nicolas-Molina
Univ de Murcia
Phone: 34648104533
fnicolas@um.es

Eva-Maria Niehaus
University of Muenster
Phone: 4.9251832499e+011
e.niehaus@uni-muenster.de

Jakob B Nielsen
DTU Systems Biology
Phone: 45 45252657
jbn@bio.dtu.dk

Michael L Nielsen
Novozymes
Phone: +45 26805119
mlxn@novozymes.com

Maria Fernanda Nieto-Jacobo
Lincoln University
Phone: +64 3 321 8113
maria.nieto-jacobo@lincoln.ac.nz

Bart Nieuwenhuis
Lab of Genetics, WUR
Phone: 31641125774
bpsnieuw@gmail.com

Marie Nishimura
Natl Inst Agrobiol Sciences
Phone: 81 2-838-8461
marie@affrc.go.jp

Clarissa Jane Nobile
UCSF
Phone: (415) 476-8097
Clarissa.Nobile@ucsf.edu

Yuhei Nogami
Toyo University
Phone: -9493
s39101200242@toyo.jp

Filomena A Nogueira
University of Aberdeen
Phone: 4.4758376676e+011
r01mfd0@abdn.ac.uk

Steffen Nordzieke
Ruhr-University Bochum
Phone: 4.9234322566e+011
steffen.nordzieke@rub.de

LIST OF PARTICIPANTS

Olga Novikova
University at Albany
Phone: 859-940-5160
novikova@albany.edu

Minou Nowrousian
Ruhr University Bochum
Phone: 49 234-3224588
minou.nowrousian@ruhr-uni-bochum.de

Donald L Nuss
University of Maryland
Phone: (240) 314-6218
dnuss@umd.edu

Kristiina Nygren
Swedish Univesity of Agricultural Sciences
Phone: (46) 734-226900
kristiina.nygren@gmail.com

Richard J O'Connell
INRA-BIOGER
Phone: 49 2215062311
oconnel@mpipz.mpg.de

Céline M O'Gorman
University of Nottingham
Phone: -8232112
celine.ogorman@nottingham.ac.uk

Berl R Oakley
Univ Kansas
Phone: (785) 864-8170
boakley@ku.edu

Ken Oda
Univ Georgia
Phone: (706) 542-6026
koda@plantbio.uga.edu

Yeon Y Oh
NCSU
Phone: (919) 513-0167
yoh2@ncsu.edu

Ayumi Ohba
Tohoku university
Phone: -9561
ayumiohba@gmail.com

Mana Ohkura
University of AZ
Phone: 520-342-4794
manabanana@email.arizona.edu

Robin A Ohm
DOE Joint Genome Institute
Phone: 925- 927-2546
RAOhm@lbl.gov

Shuichi Ohsato
Meiji University
Phone: -8710
ohsato@gmail.com

Rodrigo A Olarte
NCSU
Phone: 919 513-4867
raolarte@ncsu.edu

Vianey G Olmedo-Monfil
Universidad de Guanajuato
Phone: 52 4737320006
vianey_olmedo@yahoo.com.mx

Carsten L Olsen
Novozymes A/S
Phone: +45 30776013
cols@novozymes.com

Ake Olson
SW Univ Agricultural Sci
Phone: +46 18 671876
ake.olson@slu.se

Ryoko Oono
Duke Univ. & NC State Univ.
Phone: 5073696394
ryoko.oono@duke.edu

Marc J Orbach
Univ Arizona
Phone: (520) 621-3764
orbachmj@ag.arizona.edu

Miriam Oses-Ruiz
University of Exeter
Phone: 440-1392264689
mo256@exeter.ac.uk

Therése Oskarsson
Carlsberg Laboratory
Phone: 0046 70 8898397
tos@crc.dk

Stephen A Osmani
Ohio State Univ
Phone: (614) 247-6791
osmani.2@osu.edu

Jean-Paul J Ouedraogo
Institut of Biology
Phone: 003171 5274914
jpaul_oued@yahoo.fr

Shouqiang Ouyang
University of California, Riverside
Phone: 951-827-3190
souyang@ucr.edu

Catherine Alisha Owensby
Oregon State University
Phone: 5417375284
owensbyc@science.oregonstate.edu

Javier Palma-Guerero
UC Berkeley
Phone: 510-643-2546
jpalma@berkeley.edu

Marja Paloheimo
Roal Oy
Phone: 358 9-29042-128
marja.paloheimo@roal.fi

Franck P Panabieres
INRA-ISA
Phone: (+33) 4 92 38 65 18
franck.panabieres@sophia.inra.fr

Iovanna Pandelova
Oregon State University
Phone: (541)7372234
pandeloi@science.oregonstate.edu

Ae Ran Park
Seoul National Univ
Phone: 82 2-8804681
arpark@snu.ac.kr

HEESOO PARK
University of Wisconsin Madison
Phone: 608-263-6830
hpark48@wisc.edu

Jaejin Park
Seoul National University
Phone: -5484
asdfppp2@gmail.com

Joohae Park
Institute of Biology Leiden
Phone: 31715275072
joohae@gmail.com

Seung-Moon Park
Chonbuk Natl Univ
Phone: 82 63-270-4311
smpark@moak.chonbuk.ac.kr

Sook-Young Park
Seoul National University
Phone: 82-2-880-4635
sookyp@gmail.com

Matias Pasquali
CRP GABRIEL LIPPMANN
Phone: 352 470261436
matias.pasquali@gmail.com

Ruvini U. Pathirana
University of Nebraska - Lincoln
Phone: 4028407086
ruvini@huskers.unl.edu

Aleksandrina Patyshakuliyeva
CBS-KNAW
Phone: + 31 (0)30 2122600
a.patyshakuliyeva@cbs.knaw.nl

Linda Paun
Christian-Albrechts-University
Phone: 4.9431880551e+011
lpaun@bot.uni-kiel.de

Teresa E Pawlowska
Cornell Univ
Phone: (607) 254-8726
tep8@cornell.edu

Kabir G Peay
Stanford University
Phone: 650-723-0552
kpeay@stanford.edu

Miguel Penalva
CSIC
Phone: 3491837 3112
penalva@cib.csic.es

Gervette M Penny
St. George's University
Phone: 14734564404
gpenny001@hotmail.com

Daniel Penselin
Institute of Plant Biochemistry
Phone: +49 (0) 345 55821431
Daniel.Penselin@ipb-halle.de

Nalu Peres
Univ of Sao Paulo
Phone: 55 16 3602 3224
nalu@usp.br

Jose C Perez
UCSF
Phone: 415-476-8097
jperez@ucsf.edu

Jose Perez-Martin
Centro Natl de Biotechnology
Phone: 34 91-585-4704
jose.perez@csic.es

Mike Perlin
Univ Louisville
Phone: (502) 852-5939
mhperl01@louisville.edu

Peter Philippsen
Univ Basel
Phone: 41 61-2671480
peter.philippsen@unibas.ch

Birgit Piechulla
University of Rostock
Phone: 0049 381 4986130
birgit.piechulla@uni-rostock.de

Sebastian Pilsyk
Inst of Biochem & Biophysics PAS
Phone: 48 22-592-1209
Seba@ibb.waw.pl

Marta J Piotrowska
Scotland's Rural College
Phone: 4.413153542e+011
marta.piotrowska@sruc.ac.uk

John W Pitkin
Monsanto
Phone: (636) 737-5959
john.w.pitkin@monsanto.com

Michael Plamann
Univ Missouri, Kansas City
Phone: (816) 235-2593
plamannm@umkc.edu

David F Plaza
ETH Zurich
Phone: +41 44 632 49 24
david.plaza@micro.biol.ethz.ch

Clemence Plissonneau
INRA
Phone: 130814573
clemence.plissonneau@versailles.inra.fr

Kim M Plummer
La Trobe Univ
Phone: 61 3-9479-2223
k.plummer@latrobe.edu.au

Istvan Pocsi
Univ of Debrecen
Phone: 3652 512900
ipocsi@gmail.com

Stefanie Poeggeler
Georg-August University
Phone: 49 551-3913930
spoegge@gwdg.de

Laetitia Poidevin
Univ. of California, Riverside
Phone: (951) 522-2025
laetitia@ucr.edu

LIST OF PARTICIPANTS

Nadia Ponts
INRA
Phone: -57122466
nadia.ponts@bordeaux.inra.fr

Stephan Poppe
Max Planck Institute Marburg
Phone: 49 642 1178632
poppeste@mpi-marburg.mpg.de

Nathan Portier
Union Biometrica, Inc.
Phone: 508-893-3115
kbarnhart@unionbio.com

Joanna Potrykus
University of Aberdeen
Phone: +44 1224 437 478
j.potrykus@abdn.ac.uk

Nathalie Poussereau
Université Lyon I
Phone: 33683184278
nathalie.poussereau@univ-lyon1.fr

Robert J Pratt
DuPont Industrial Biosciences
Phone: (650) 846-7500
robert.pratt@dupont.com

Bert-Ewald Priegnitz
TU Braunschweig
Phone: 0531 391 5787
B-E.Priegnitz@tu-bs.de

Anne Pringle
Harvard University
Phone: (617) 496-9707
pringle@oeb.harvard.edu

Robert H Proctor
NCAUR/ARS/USDA
Phone: (309) 681-6380
proctorh@ars.usda.gov

Dov B Prusky
Agricultural Research Org
Phone: 972-3-9683880
dovprusk@volcani.agri.gov.il

Patricia J Pukkila
Univ North Carolina
Phone: (919) 966-5576
pukkila@unc.edu

Peter J Punt
TNO
Phone: 31 88-8661728
peter.punt@tno.nl

Terhi J Puranen
Roal Oy
Phone: 358 9 2904 2127
terhi.puranen@roal.fi

Xiaodong Qi
Arizona State University
Phone: 480-965-1928
xiaodong@asu.edu

Katrin Quester
CICESE
Phone: +52 646 1750500
quester@cicese.edu.mx

Franziska Rabe
MPI Marburg
Phone: 49 64211 78533
franziska.rabe@mpi-marburg.mpg.de

Roman Rabinovich
DuPont Industrial Biosciences
Phone: 650-245-9059
Roman.Rabinovich@dupont.com

Mostafa Rahnama
University of Auckland
Phone: 64220863333
mrah233@aucklanduni.ac.nz

Marcelo HS Ramada
Brasilia University
Phone: 5.5629980492e+011
marceloramada@gmail.com

Marilee A Ramesh
Roanoke College
Phone: (540) 375-2464
ramesh@roanoke.edu

Christof Rampitsch
Agriculture & Agrifood Canada
Phone: 204 983-2385
chris.rampitsch@agr.gc.ca

Dustin Parker Rand
Arizona State University
Phone: (480) 965-1928
dustin.rand@asu.edu

Carlene A Raper
University of Vermont
Phone: 802-660-4809
carlene.raper@uvm.edu

Chad A Rappleye
Ohio State University
Phone: (614) 247-2718
rappleye.1@osu.edu

Davide Ravasio
Carlsberg Laboratory
Phone: +45 33275235
davide.ravasio@gmail.com

Nick Read
University of Edinburgh
Phone: 44 131-650-5335
Nick.Read@ed.ac.uk

Samara L Reck-Peterson
Harvard Med Sch
Phone: 617-432-7178
reck-peterson@hms.harvard.edu

Amey Redkar
MPI for Terrestrial Microbiology
Phone: 49 64211 78610
amey.redkar@mpi-marburg.mpg.de

Regina S Redman
Adaptive Symbiotic Technologies
Phone: (206) 661-8064
ast-reginaredman@comcast.net

Morgann C Reilly
University of California, Berkeley
Phone: 510-666-2559
mcreilly@berkeley.edu

Stefanie G Reissmann
Max-Planck Inst
Phone: 4.9642117185e+012
reissmas@mpi-marburg.mpg.de

Ana Rems
Technical University of Denmark
Phone: 4542570887
anare@bio.dtu.dk

Martijn Rep
University of Amsterdam
Phone: 31 20-525-7764
m.rep@uva.nl

Timothy Repas
University of Saskatchewan
Phone: 3062410474
tsr852@mail.usask.ca

Natalia Requena
Karlsruhe Institute of Technology
Phone: 49-721-60844626
natalia.requena@kit.edu

Hannah T Reynolds
University of Akron
Phone: 919-698-2846
hannah.t.reynolds@gmail.com

Hashim H Reza
Genome Research Centre
Phone: 9.1960139119e+011
dreamzhashim908@gmail.com

Robert W Riley
Lawrence Berkeley National Lab
Phone: 925-296-5797
rwriley@lbl.gov

Meritxell Riquelme
CICESE
Phone: 52 646-1750500
riquelme@cicese.mx

Barbara Robbertse
NCBI
Phone: (301) 594-5068
robberts@ncbi.nlm.nih.gov

Angélica Mariana Robledo Briones
Cinvestav Unidad Irapuato
Phone: +52 (462) 623 96 00
jruiz@cinvestav.mx

Rusty J Rodriguez
Symbiogenics
Phone: (206) 661-8065
rjrodriguez@symbiogenics.org

Antonis Rokas
Vanderbilt University
Phone: (615) 936-3892
antonis.rokas@vanderbilt.edu

M. Isabel G Roncero
University of Cordoba
Phone: +34 957218981
roncero@uco.es

Antonio Rossi
FMRP-USP
Phone: 55 16-36023112
anrossi@usp.br

Marika Rossi
National Research Council
Phone: 3.9011397792e+011
m.rossi@ivv.cnr.it

Thierry Rouxel
INRA
Phone: 33 1 30 81 45 73
rouxel@versailles.inra.fr

John C Royer
DSM
Phone: 617 913-5167
John.royer@dsm.com

Jason J Rudd
Rothamsted Research
Phone: +44 (0)1582 763133
jason.rudd@rothamsted.ac.uk

Carmen Ruger-Herreros
Univ de Sevilla
Phone: 34954556473
carmenruger@us.es

Farhana Runa
North Carolina State University
Phone: 626-325-4067
fruna@ncsu.edu

Jesper H Rung
Novozymes
Phone: 0045 44460349
rung@novozymes.com

Lauren S Ryder
Univ Exeter
Phone: 44 0-1392 264689
l.s.ryder@ex.ac.uk

Johanna Rytioja
University of Helsinki
Phone: 3.5891915932e+011
johanna.rytioja@helsinki.fi

Elzbieta Rzeszutek
Royal Institute of Technology
Phone: +46-(0)8-5537-8448
elrz@kth.se

Teppo - Rämä
University of Tromso, Norway
Phone: 5419744703
teppo.rama@uit.no

Matthew S Sachs
Texas A&M Univ
Phone: (979) 845-5930
msachs@mail.bio.tamu.edu

Divya Sain
Univ California, Riverside
Phone: (951) 756-5449
dsain001@ucr.edu

Wasin Sakulkoo
University of Exeter
Phone: -7884157152
ws254@exeter.ac.uk

LIST OF PARTICIPANTS

Asaf A Salamov
DOE Joint Genome Inst
Phone: (925) 296-5782
aasalamov@lbl.gov

Marketa Samalova
University of Oxford
Phone: 4.401865275e+012
marketa.samalova@plants.ox.ac.uk

Eddy F Sanchez-Leon
CICESE
Phone: 52 646 1750500
eddy.san.leon@gmail.com

Andrea Sanchez-Vallet
Wageningen University
Phone: 0031 (0)317485325
andrea.sanchezvallet@wur.nl

Björn Sandrock
Philipps-University Marburg
Phone: +49 6421 2827080
sandrock@staff.uni-marburg.de

Dominique Sanglard
Univ of Lausanne and Hosp Ctr
Phone: 41213144883
Dominique.Sanglard@chuv.ch

Hiroaki Sano
Forestry and Forest Products Research Institute
Phone: +81 29 829 8279
sanoh@affrc.go.jp

Marcia R M Saraiva
University of Aberdeen
Phone: 4.4787902256e+011
m.saraiva@abdn.ac.uk

Oezlem Sarikaya Bayram
Inst. of Microbiology and Genet
Phone: 49551393821
osarika@gwdg.de

Mohammad N SAROWAR
Institute of Medical Sciences
Phone: 7741511109
r01mns11@abdn.ac.uk

Kengo Sasaki
Tokyo Univ. of Science
Phone: -16194
j6412624@ed.tus.ac.jp

Yuki SATOH
Hokkaido Univ.
Phone: -3138
yuki-s@chem.agr.hokudai.ac.jp

Charles W Saunders
Procter & Gamble
Phone: (513) 622-4982
saunders.cw@pg.com

Sven J Saupe
CNRS
Phone: 33 5-56999027
sven.saupe@ibgc.u-bordeaux2.fr

Barry J Saville
Trent University
Phone: (705) 748-1011
barrysaville@trentu.ca

Gabriel Scalliet
Syngenta
Phone: +41 62 8660122
gabriel.scalliet@syngenta.com

Patrick C Schacht
Univ California, Riverside
Phone: (951) 823-0017
pscha001@ucr.edu

Christopher W Schadt
Oak Ridge National Laboratory
Phone: 865.576.3982
schadtcw@ornl.gov

Katja Schaefer
University of Cordoba
Phone: 34957212421
k_scha11@hotmail.de

Wilhelm Schaefer
University Hamburg
Phone: 0049-4042816266
wilhelm.schaefer@googlemail.com

Les L Scharfenstein
Southern Regional Research Center
Phone: 504-286-4374
les.scharfenstein@ars.usda.gov

Kirstin Scherlach
Leibniz Institute, HKI
Phone: + 49 3641 5321034
kirstin.scherlach@hki-jena.de

Josua Schinke
Georg-August-University
Phone: +49 17623974760
jschink2@gwdg.de

Kerstin Schipper
University Düsseldorf
Phone: 0049-211-8110451
kerstin.schipper@hhu.de

Jan Schirawski
RWTH Aachen University
Phone: 49-241-8026616
jan.schirawski@rwth-aachen.de

Morten Schiøtt
University of Copenhagen
Phone: +45 35321255
mschiott@bio.ku.dk

Georg Schmidt
VTT
Phone: +358 40 723 1815
georg.schmidt@vtt.fi

Sarah Maria Schmidt
University of Amsterdam
Phone: 31623236526
s.m.schmidt@uva.nl

Stefanie S Schmieder
ETH Zurich
Phone: +41 44 632 49 24
sschmied@ethz.ch

Monika Schmoll
Austrian Institute of Technology
Phone: 43 664 88390594
monika.schmoll@ait.ac.at

Klaas Schotanus
Max-Planck Inst.
Phone: +49 6421 178630
klaas.schotanus@mpg-marburg.mpg.de

Timo Schuerg
UC Berkeley
Phone: 5105026980
tschuerg@berkeley.edu

Julia Schumacher
Purdue University
Phone: 4.9178600024e+011
julia.schumacher@wwu.de

Barry Scott
Massey Univ
Phone: 6463505543
d.b.scott@massey.ac.nz

Frank J.J. Segers
CBS-KNAW Fungal Biodiversity Centre
Phone: +31(0)302122669
f.segers@cbs.knaw.nl

Stephan S Seiler
University Freiburg
Phone: 49 551-393777
sseiler@gwdg.de

Marc-André Selosse
Centre d'Ecologie Fonctionelle et Evolutive (CNRS)
Phone: +33 607123418
ma.selosse@wanadoo.fr

Dominik Senftleben
Friedrich-Schiller-Universitat
Phone: 49 3641 949399
dominik.senftleben@uni-jena.de

Ane Sesma
CBGP/ Univ Politécnica de Madrid
Phone: +34 91 336 4593
ane.sesma@upm.es

Firoz Shah
Lund University
Phone: 46462223757
Firoz.Shah@biol.lu.se

Shermineh Shahi
University of Amsterdam - SILS
Phone: (0031) 20-525-8415
s.shahi@uva.nl

Viplendra P.S. Shakya
UMKC
Phone: (201)993-7038
vps7y4@mail.umkc.edu

Amir Sharon
Tel Aviv University
Phone: 972 36406741
amirsh@ex.tau.ac.il

Sharpee C Sharpee
NC State University
Phone: 608-576-8890
wcsharpe@ncsu.edu

Brian D Shaw
Texas A&M Univ
Phone: (979) 862-7518
bdshaw@tamu.edu

Danyu Shen
Oregon State University
Phone: 541-602-9583
shendanyu9@gmail.com

Kuwata Shigeru
Meiji University
Phone: -7933
kuwata@meiji.ac.jp

Jason Shiller
La Trobe University
Phone: 61426248488
jshiller@students.latrobe.edu.au

Patrick Shiu
University of Missouri
Phone: 573-884-0020
shiup@missouri.edu

Jun-ya Shoji
The Samuel Roberts Noble Foundation
Phone: 5802246968
jyshoji@yahoo.co.jp

Bhushan Shrestha
Rural Development Administration
Phone: -11361
bhushan.shrestha@gmail.com

Christian Sieber
Helmholtz-Zentrum München
Phone: +49 (0)89 3187 3577
christian.sieber@helmholtz-muenchen.de

Ulrike Siegmund
University of Muenster
Phone: 4.9521832499e+011
ulrike.siegmund@uni-muenster.de

Anita Sil
Univ California, San Francisco
Phone: (415) 502-1805
sil@cgl.ucsf.edu

Larissa Silva
University of Sao Paulo
Phone: 55 (16) 3602-3078
larissa.gomes@usp.br

Roberto N. Silva
University of São Paulo
Phone: 5.5163602326e+011
rsilva@fmrp.usp.br

Paul Skatrud
Monsanto
Phone: 636-737-7082
paul.l.skatrud@monsanto.com

Dominique A Skovlund
Novozymes A/S
Phone: +45 44 465892
dau@novozymes.com

Stephanie L Slinski
University of Pretoria
Phone: 27124206937
stephanie.slinski@fabi.up.ac.za

Jason Slot
Vanderbilt University
Phone: (615) 266-1433
jason.c.slot@vanderbilt.edu

LIST OF PARTICIPANTS

Francis Smet
Union Biometrica
Phone: 3214570628
fsmet@unionbio.com

Kristina M Smith
Oregon State Univ
Phone: (541) 737-4399
smitkris@science.oregonstate.edu

Karen M Snetselaar
St Joseph's Univ
Phone: (610) 660-1826
ksnetsel@sju.edu

Peter S Solomon
Australian National University
Phone: -1391
peter.solomon@anu.edu.au

James TG Solomons
SUNY Buffalo
Phone: 716 829 2091
jgsolomo@buffalo.edu

Benesh M Somai
Nelson Mandela Metropolitan University
Phone: 27-71-0211-090
benesh.somai@nmmu.ac.za

HOKYOUNG SON
Seoul National Univ
Phone: 82 2-8804681
hogongi7@snu.ac.kr

Teruo Sone
Hokkaido Univ
Phone: 81 11-706-2502
sonet@chem.agr.hokudai.ac.jp

Vicky Sophianopoulou
Biosciences and applications
Phone: 30 210 6503602
vicky@bio.demokritos.gr

Alexandra Soukup
UW-Madison
Phone: 6082621957
soukup@wisc.edu

Jessica Soyer
INRA
Phone: 33-130814593
jessica.soyer@versailles.inra.fr

Joseph W Spatafora
Oregon State Univ
Phone: (541) 737-5304
spatafoj@bcc.orst.edu

Richard Splivallo
University of Goettingen
Phone: 4.9176649314e+012
richard.splivallo@a3.epfl.ch

Joseph Spraker
UW-Madison
Phone: (608)262-1958
jspraker@wisc.edu

Jason Stajich
Univ California, Riverside
Phone: (951) 827-2363
jason.stajich@ucr.edu

Claire Stanley
Institute of Chemical and Bioengineering
Phone: 41774262541
claire.stanley@chem.ethz.ch

Trevor Starr
UC Berkeley
Phone: 5108470789
t_starr@berkeley.edu

Emma T Steenkamp
University of Pretoria
Phone: 27 12 420 3262
emma.steenkamp@up.ac.za

Matthias G Steiger
ACIB GmbH / BOKU
Phone: 4313180078
matthias.steiger@boku.ac.at

Gero Steinberg
Univ Exeter
Phone: 0044-7717-243650
G.Steinberg@exeter.ac.uk

Andrei S Steindorff
Brasilia University
Phone: 55-6291037954
andreistecca@gmail.com

Karen Stephenson
University of Dundee
Phone: 4.413823863e+011
k.stephenson@dundee.ac.uk

Ioannis Stergiopoulos
UC Davis
Phone: (530) 400-9802
istergiopoulos@ucdavis.edu

Eva H Stukenbrock
Max Planck Institute
Phone: 0049 6421 178 630
eva.stukenbrock@mpi-marburg.mpg.de

Joy E Sturtevant
LSUHSC
Phone: (504) 568-6116
jsturt@lsuhsc.edu

Martina A Stöckli
ETH Zurich
Phone: 0041 44 632 45 53
smartina@micro.biol.ethz.ch

Gopal Subramaniam
Agriculture Canada
Phone: (613) 759-7619
subramaniamra@agr.gc.ca

Peter E Sudbery
Sheffield Univ
Phone: 114 2226186
p.sudbery@shef.ac.uk

Mallory Suhr
University of Nebraska-Lincoln
Phone: 402-641-5999
msuhr@huskers.unl.edu

Sheng Sun
Duke University Medical Center
Phone: (919) 684-3036
sheng.sun@duke.edu

Johanna Takach
The Noble Foundation
Phone: (580) 224-6852
jetakach@noble.org

Tsukasa Takahashi
Toyo University
Phone: -9581
dx0900031@toyo.jp

Norio Takeshita
Karlsruhe Institute of Technology
Phone: -45913
norio.takeshita@kit.edu

Jennifer M Talbot
Stanford University
Phone: 6178693715
jmtalbot@stanford.edu

Nick J Talbot
Univ Exeter
Phone: 44 1392-72-3006
n.j.talbot@exeter.ac.uk

Koichi Tamano
AIST
Phone: -10246
tamano-k@aist.go.jp

Kaeling Tan
Harvard Medical School
Phone: (617) 432-7177
kaeling_tan@hms.harvard.edu

Kar-Chun Tan
Curtin University
Phone: 61892669916
Kar-Chun.Tan@curtin.edu.au

Mizuki Tanaka
Tohoku University
Phone: -9561
mizu-t@biochem.tohoku.ac.jp

Shuji Tani
Osaka Prefecture Univ
Phone: 81-72-254-9466
shuji@biochem.osakafu-u.ac.jp

John W Taylor
Univ California, Berkeley
Phone: (510) 642-5366
jtaylor@berkeley.edu

Samantha Taylor
Oregon State University
Phone: 304-942-6007
samantha.taylor@oregonstate.edu

Ines Teichert
Ruhr University Bochum
Phone: 4.9234322497e+011
ines.teichert@rub.de

Nora Temme
KWS SAAT AG
Phone: 49 5561 311 1141
nora.temme@kws.com

Hiroshi Teramoto
Novozymes Japan Ltd.
Phone: +81 43-296-6770
hite@novozymes.com

Eeva Terhonen
Helsinki University
Phone: +35 40 7510773
eeva.terhonen@helsinki.fi

Bart P Thomma
Wageningen University
Phone: 0031-317-484536
bart.thomma@wur.nl

Ashley Thompkins
Coker College
Phone: (843) 241-4107
ashley.thompkins@coker.edu

Dawn Thompson
Broad Institute
Phone: 6177147763
dawnt@broadinstitute.org

Darren Thomson
Abderdeen University
Phone: 7980405654
d.thomson@abdn.ac.uk

Britta A. M. Tillmann
Philipps-University Marburg
Phone: 4.9642128271e+012
britta-tillmann@gmx.de

Richard B Todd
Kansas State University
Phone: (785) 532-0962
rbtodd@ksu.edu

Su San Toh
University of Louisville
Phone: 5028525939
susan.toh@louisville.edu

Marie Tollot
MPI Marburg
Phone: 49 64211 78571
marie.tollot@mpl-marburg.de

Xiaoxue Tong
Aalborg University
Phone: (+45) 50359928
xiat@bio.aau.dk

Stefano F Torriani
ETH Zurich
Phone: 41 (0)44 632 38 48
stefano.torriani@agrl.ethz.ch

Frances Trail
Michigan State Univ
Phone: 517-432-2939
trail@msu.edu

Adrian Tsang
Concordia Univ
Phone: (514)848-2424 x3405
adrian.tsang@concordia.ca

Bettina Tudzynski
Westf Wilhelms Univ Munster
Phone: 49 251-832-4801
bettina.tudzynski@uni-muenster.de

Paul Tudzynski
West F Wilhelms Univ Muenster
Phone: 49 251-832-4998
tudzyns@uni-muenster.de

LIST OF PARTICIPANTS

Anders P V Tunlid
Ecology
Phone: 46 46-2223757
anders.tunlid@mbioekol.lu.se

Gillian Turgeon
Cornell Univ
Phone: (607) 254-7458
bgt1@cornell.edu

Massimo Turina
CNR
Phone: 3.9011397792e+011
m.turina@ivv.cnr.it

David Turra
University of Cordoba
Phone: 0034 957 218981
ge3tutud@uco.es

Brett M Tyler
Oregon State University
Phone: 541-737-3347
brett.tyler@oregonstate.edu

Georgios Tzelepis
SLU
Phone: 4618671835
georgios.tzelepis@slu.se

Hiroaki Udagawa
Novozymes Japan
Phone: -432966689
huda@novozymes.com

Jessie Uehling
Duke University
Phone: 2088712701
jku@duke.edu

Srijana Upadhyay
Texas A&M University
Phone: (979) 845-7259
srijanaupadhyay@tamu.edu

Martin Urban
Rothamsted Research
Phone: (44) 01582-763133
martin.urban@bbsrc.ac.uk

Barbara Valent
Kansas State Univ
Phone: (785) 532-2336
bvalent@ksu.edu

Clarissa Xavier Res Valim
FMRP-USP
Phone: 55-16-3602-3143
clarissavalim@hotmail.com

Mari Valkonen
VTT
Phone: 358 20 722-5825
mari.valkonen@vtt.fi

Peter van Dam
University of Amsterdam - SILS
Phone: 31205258412
p.vandam@uva.nl

Guido Van den Ackerveken
Utrecht University
Phone: 31302533013
g.vandenackerveken@uu.nl

Charlotte van der Does
University of Amsterdam
Phone: 31205257764
H.C.vanderdoes@uva.nl

Jan van Kan
Wageningen Univ
Phone: 31 317 483126
jan.vankan@wur.nl

Martin van Leeuwen
CBS-KNAW Fungal Biodiversity Centre
Phone: 0031 (0)30 621666
r.vanleeuwen@cbs.knaw.nl

Hans D VanEtten
Univ Arizona
Phone: (520) 621-9355
vanetten@ag.arizona.edu

Elizabeth A Veal
Newcastle Univ
Phone: 44 191-222-7596
e.a.veal@ncl.ac.uk

Sudhagar Veerabadran Balasundaram
University of Oslo
Phone: 4794824403
sudhagar@bio.uio.no

Claire Veneault-Fourrey
Lorraine University / INRA
Phone: 33 (0)3 83 39 40 81
claire.fourrey@univ-lorraine.fr

Surbhi Verma
University of Missouri-Kansas City
Phone: 1-816-235-1553
svb66@mail.umkc.edu

Paul Verweij
UMC ST RADBOUD
Phone: -3614349
P.verweij@mmb.umcn.nl

Muriel Viaud
INRA
Phone: 33 1 30 81 45 68
viaud@versailles.inra.fr

Rytas Vilgalys
Duke University
Phone: (919) 660-7361
fungi@duke.edu

Stela Virgilio
Instituto de Quimica, UNESP
Phone: 55-16-33019804
stelav5@yahoo.com.br

Hans Visser
Dyadic Nederland BV
Phone: 31 317 465457
hvisser@dyadic.nl

Ido Vlaardingerbroek
University of Amsterdam
Phone: 31205258414
i.vlaardingerbroek@uva.nl

Christian A Voigt
Biocenter Klein Flottbek
Phone: -42816322
voigt@botanik.uni-hamburg.de

Susanne Voigt
Univ. of Hamburg
Phone: 4940 4281 6331
voigt@botanik.uni-hamburg.de

Christine R Voisey
AgResearch
Phone: 61 6-3518080
christine.voisey@agresearch.co.nz

Johannes Wagener
University of Munich
Phone: -6708778
johannes.wagener@med.uni-muenchen.de

Andrea Walther
Carlsberg Lab
Phone: +45 3327 5391
andrea.walther@carlsberglab.dk

Jonathan Walton
Michigan State University
Phone: 517-353-4885
walton@msu.edu

Chen fang Wang
Northwest A&F University
Phone: 86-029-87081270
wangchenfang@nwsuaf.edu.cn

Chengshu Wang
Shanghai Institutes for Biological Sciences, CAS
Phone: -54924092
cswang@sibs.ac.cn

Clay Wang
Univ Southern California
Phone: (323) 442-1670
clayw@usc.edu

Fengfeng Wang
Utrecht University
Phone: +31 30 2533632
F.wang@uu.nl

Guanghui Wang
Northwest A & F University
Phone: 765-418-6246
wgh2891458@163.com

Linqi Wang
Texas A&M University
Phone: (979) 845-7259
lqwang@bio.tamu.edu

MING WANG
UC, Riverside
Phone: 951-961-1183
mwang012@ucr.edu

Ping Wang
Res Inst Children/LSU
Phone: (504) 896-2700
pwang@chnola-research.org

Qunqing Wang
OREGON STATE UNIVERSITY
Phone: 541-737-3679
qunqing.wang@oregonstate.edu

Ting-Fang Wang
Academia Sinica
Phone: 886-2-27899188
tfwang@gate.sinica.edu.tw

Xuying Wang
Duke Univ
Phone: (205) 482-0213
xuying.wang@duke.edu

Yizhou Wang
Univ. of CA, Riverside
Phone: 951-275-6883
ywang039@ucr.edu

Yuanchao Wang
Nanjing Agricultral University
Phone: 86-25-84399071
wangyc@njau.edu.cn

Michael Ward
DuPont Industrial Biosciences
Phone: (650) 846-5850
mward@genencor.com

Todd J Ward
USDA ,ARS,NCAUR
Phone: (309) 681-6394
wardtj@ncaur.usda.gov

Stephan Wawra
Univ Aberdeen
Phone: 44 1224 437329
s.wawra@abdn.ac.uk

Roland Wedlich-Soldner
MPI of Biochemistry
Phone: +49 89 8578 3410
wedlich@biochem.mpg.de

Harshini C Weerasinghe
The University of Melbourne
Phone: 61411362559
h.weerasinghe@student.unimelb.edu.au

Michaela Wehr
Philipps University
Phone: 64212827078
Michaela_Wehr@web.de

Arne Weiberg
UCR
Phone: 9512757333
aweiberg@ucr.edu

Martin Weichert
TU Braunschweig
Phone: -6660
m.weichert@tu-bs.de

Jurgen W Wendland
Carlsberg Laboratory
Phone: 45 3327 5230
jww@crc.dk

Emily A Whiston
U.C. Berkeley
Phone: (510) 642-8441
whiston@berkeley.edu

Graham Whyteside
DSM
Phone: 31152792118
graham.whyteside@dsm.com

Phillipp Wiemann
Univ. Münster
Phone: 49 251 8324806
philipp.wiemann@uni-muenster.de

LIST OF PARTICIPANTS

Aric E Wiest
Univ Missouri, KC
Phone: (816) 235-6485
wiesta@umkc.edu

Richard A Wilson
University of Nebraska-Lincoln
Phone: 4024722156
rwilson10@unl.edu

Brenda D Wingfield
University of Pretoria
Phone: 27 12 420 3946
brenda.wingfield@up.ac.za

Hua Z Wise
Oregon State University
Phone: (512)9213055
zhanghua72@gmail.com

Thomas J. Wolpert
Oregon State University
Phone: 541-737-5293
wolpertt@science.oregonstate.edu

Koon Ho Wong
Harvard Medical School
Phone: 6174322105
koonho_wong@hms.harvard.edu

Jennifer R Wortman
Broad Institute
Phone: 240-441-9094
jwortman@broadinstitute.org

Dongliang Wu
Cornell University
Phone: 607-257-2683
dongliangwu@yahoo.com

Jiayao Wu
Helsinki University
Phone: 3.580417058e+012
jiayao.wu@helsinki.fi

Timon Wyatt
CBS Fungal Biodiversity Centre
Phone: 31302122600
timonwyatt@gmail.com

Xin Xiang
USUHS
Phone: (301) 295-0000
xxiang@usuhs.mil

Yi Xiong
Univ California, Berkeley
Phone: 510-666-2559
yi-xiong@berkeley.edu

Jin-Rong Xu
Purdue Univ
Phone: (765) 496-6918
jinrong@purdue.edu

Jun Xu
P&G
Phone: 5136220290
xu.j.1@pg.com

Wenjie Xu
Carnegie Mellon University
Phone: 8587228372
wxu7206@gmail.com

Chun-Hsiang Yang
National Ilan University
Phone: 8.8692870579e+011
philosophyroy@hotmail.com

Dong-Hoon Yang
Yonsei University
Phone: 82-70-7578-1596
garaman99@nate.com

Moon S Yang
Chonbuk National Univ
Phone: 82 63-270-3339
mskyang@jbnu.ac.kr

Oded Yarden
Hebrew University of Jerusalem
Phone: 972 8 9489298
Oded.Yarden@huji.ac.il

Wenwu Ye
Nanjing Agricultral University
Phone: 86-25-84399071
2010202018@njau.edu.cn

Lakshmi Preethi Yerra
UNL
Phone: 201-925-8484
s-lyerra1@unl.edu

Mihwa Yi
Kansas State University
Phone: 785-532-2337
mihwa@ksu.edu

Wang Yonglin
Beijing Forestry University
Phone: 13488765326
ylwang@bjfu.edu.cn

Carolyn A Young
Noble Foundation
Phone: (580) 224-6860
cayoung@noble.org

Jaehyuk Yu
Univ Wisconsin, Madison
Phone: (608) 262-4696
jyu1@wisc.edu

Sung-Hwan Yun
Soonchunhyang Univ
Phone: 82 41-5301288
sy14@sch.ac.kr

Ivo Zadra
SANDOZ GmbH
Phone: +43 (0)5338 200 3509
ivo.zadra@sandoz.com

Donald R Zak
University of Michigan
Phone: 734763-4991
drzak@umich.edu

Dongxiu Zhang
UM IBBR
Phone: 240-314-6497
dzhan5@umd.edu

Jian Zhang
Nanjing Agricultural University
Phone: (86) 15850560269
zhangjian106@hotmail.com

Ning Zhang
Rutgers Univ
Phone: 8145743939
zhang@aesop.rutgers.edu

Shizhu Zhang
Nanjing Normal University
Phone: 86 (25) 85891895
shizhuz@126.com

Ying Zhang
KarlsruheInstitute of Technology
Phone: 17655321539
fionazhangying@hotmail.com

Zhenyu Zhang
UT Southwestern Medical Center
Phone: 2149301249
zhenyuzhang86@gmail.com

Jiuhai Zhao
UC berkeley
Phone: 650-215-3209
jhzhao@berkeley.edu

Yun Zheng
Concordia University
Phone: 514-848-2424 ext4025
zhyun627@gmail.com

Shaobin Zhong
North Dakota State Univ
Phone: (701) 231-7427
shaobin.zhong@ndsu.edu

Mian Zhou
UT Southwestern Medical Center
Phone: 9729788908
mian.zhou@utsouthwestern.edu

Xiaoying Zhou
Purdue University
Phone: 765-418-6157
maidou2010@gmail.com

Kolea Zimmerman
Harvard University
Phone: 8089898031
kzimmerman@fas.harvard.edu

Miriam E Zolan
Indiana Univ
Phone: (812) 855-6694
mzolan@indiana.edu

Alga Zuccaro
Max Planck Institute
Phone: 4.964211786e+011
zuccaro.alga@mpi-marburg.mpg.de

STUDENT POSTER LIST