Get ready for infection: Transcriptional profiling reveals virulence-specific traits inside of infection cushions of Fusarium graminearum

Jorg Bormann, Marike J. Boenisch, Anika Glasenapp, Ana-Lilia Martinez Rocha, Stefan Scholten, Sebastian Piehler, Martin Münsterkötter, Ulrich Güldener, Bernard Henrissat, Marc-Henri M.-H. Lebrun, et al.

Full Abstracts

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PLENARY SESSION ABSTRACTS

Wednesday, March 18 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session I: Evolution
Chair: John Taylor

Food for thought: Cheese as an experimental ecosystem. Benjamin Wolfe, Julie Button, Marcela Santarelli, Rachel Dutton. FAS Center for Systems Biology, Harvard University, Cambridge, MA.

Tractable microbial communities are needed to bridge the gap between observations of patterns of microbial diversity and mechanisms that can explain these patterns. We developed cheese rinds as model microbial communities by characterizing *in situ* patterns of diversity and by developing an *in vitro* system for community reconstruction. Sequencing of 137 different rind communities across 10 countries revealed 24 widely distributed and culturable genera of bacteria and fungi as dominant community members. Reproducible community types formed independent of geographic location of production. Intensive temporal sampling demonstrated that assembly of these communities is highly reproducible. Patterns of community composition and succession observed *in situ* can be recapitulated in a simple *in vitro* system. Widespread positive and negative interactions were identified between bacterial and fungal community members. Cheese rind microbial communities represent an experimentally tractable system for defining mechanisms that influence microbial community assembly and function.

Invade, co-opt, and swap: Evolution of cell cycle control in Fungi and other eukaryotes. Edgar M. Medina[1], Jon Turner[2], Jan Skotheim[2], Nicolas E. Buchler[1]. 1) Department of Biology, Duke University, Durham, NC; 2) Department of Biology, Stanford University, Stanford, CA.

Cell division is an essential process that has been occurring in an uninterrupted chain for billions of years. Thus, one expects strong conservation in the regulatory network controlling the eukaryotic cell division cycle. In eukaryotes, the cell cycle control network regulates proliferation. Earlier comparison of fungi with animals suggested that a simple ancestral eukaryotic cell cycle was driven by mitotic cyclin-dependent kinase activity, while complex G1 control evolved later. Here, we examine diverse genomes to show that the last eukaryotic common ancestor already had similar G1 regulators to animals, which have been maintained in nearly all eukaryotes. In contrast, evolution along the fungal lineage was punctuated by the acquisition and entrainment of the SBF transcription factor, likely of viral origin. We propose that SBF hijacked cell cycle control by activating genes targeted by the ancestral cell cycle regulator E2F. Cell cycle evolution in the fungal ancestor proceeded through a hybrid network containing both E2F and SBF, which is still maintained in many basal fungi. Such hybrid networks may represent a common mechanism through which core regulatory networks can dramatically evolve. Chytrids and other basal fungi are in a unique transitional evolutionary position because they exhibit both fungal and animal-like features. We are currently testing whether the SBF and E2F branches of the hybrid network regulate fungal and animal-like traits in an extant Chytrid, *Spizellomyces punctatus*.

Beyond the whole genome duplication: phylogenetic evidence for an ancient inter-species hybridization in the yeast lineage. Toni Gabaldón[1,2,3], Marina Marcet-Houben[1,2]. 1) Comparative Bioinformatics, Center for Genomic Regulation, Barcelona, Barcelona, Spain; 2) Universitat Pompeu Fabra (UPF). 08003 Barcelona, Spain; 3) Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain.

Whole genome duplications have shaped the genomes of several vertebrate, plant and fungal lineages. Earlier studies have focused on establishing when these events occurred, and on elucidating their functional and evolutionary consequences, but we still lack sufficient understanding of how genome duplications first originated. We used phylogenomics to study the ancient genome duplication occurred in the yeast lineage and show compelling evidence for the existence of a coetaneous inter-species hybridization. We propose that the genome doubling was a direct consequence of this hybridization and that it served to provide stability to the recently formed allopolyploid. This scenario provides a mechanism for the origin of this ancient duplication and the lineage that originated from it, and brings a new perspective to the interpretation of the origin and consequences of whole genome duplications.

Epigenetic variation and adaptation in plant pathogenic oomycetes. Mark Gijzen, Sirjana Shrestha, Chelsea Ishmael, Kuflom Kuflu, Yun Zhang, Patrick Chapman. Agriculture and Agri-Food Canada, London, ON, Canada.

Plant pathogens and their hosts are in engaged in a struggle to control the course of infection, and whether the outcome results in disease or immunity. Pathogens secrete effectors to promote their own growth and reproduction, while host plants deploy immune receptors for surveillance and detection of the effectors. Pathogen effector proteins that trigger immunity in host plants are avirulence (Avr) factors, encoded by *Avr* genes. As the number of known *Avr* genes from oomycete plant pathogens has grown, it is apparent that transcriptional polymorphisms often cause strain-specific gain-of-virulence changes. Loss of effector gene mRNA may be due to conventional mutations that disable transcription but epigenetic changes can also underlie these polymorphisms. In fact, experiments from our laboratory and others suggest a larger role for epigenetic variation in adaptation and evolution in plant pathogenic oomycetes. Most of the evidence arises from studies on *Phytophthora sojae* and *Phytophthora infestans*, which cause root rot of soybean and late blight of potato, respectively, but observations from other species have also contributed. In *P. sojae*, epigenetic gene silencing of *Avr* effectors can be accompanied by the presence of small RNA and lead to unusual inheritance patterns. Crosses of *P. sojae* strains point to epistatic effects upon transgenerational gene silencing of *Avr* genes, and suggest adaptive interplay between conventional and epigenetic variation in *Phytophthora*. Much remains to be discovered regarding the mechanisms of epigenetic control but operationally the phenomenon helps to answer longstanding questions and supplies material for new hypotheses. Epigenetic switching of *Avr* gene expression states could explain past observations of

spontaneous virulence changes occurring in the laboratory and in the field. Epigenetic control of *Avr* genes potentially offers pathogens the ability to re-activate or recycle effectors in response to changes in host immunity or after hard selective sweeps. Evolution may favor systems that retain *Avr* genes for future use, enabling pathogens to express a complement of effectors that evades host immunity with a minimum fitness penalty.

Genome sequence evolution in experimental populations of *S. cerevisiae*. Gregory Lang. Department of Biological Sciences, Lehigh University, Bethlehem, PA.

Adaptive evolution proceeds by the fixation of beneficial mutations; each mutation advancing the population along a particular evolutionary path. However, we lack a general understanding of why particular mutations fix in a population and how the identity of fixed mutations affects subsequent evolution through non-additive genetic interactions (epistasis).

I will discuss the results from a long-term evolution experiment in yeast and the subsequent whole-genome whole-population time course sequencing of 40 of these populations as well as recent work examining the effect of ploidy on the spectrum of evolved mutations. Our results show that patterns of sequence evolution are driven by a balance between chance events, which increase stochastic variation in evolutionary outcomes, and the deterministic action of selection on individual mutations, which favors parallel evolutionary solutions in replicate populations.

Thursday, March 19 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session II: Development
Chair: Miguel Penalva

Intra-Species Phenotypic and Genotypic Variation in *C. albicans*. Matthew Hirakawa[1], Diego Martinez[2], Sharadha Sakthikumar[2], Matthew Anderson[1], Aaron Berlin[2], Sharvari Gujja[2], Quandong Zeng[2], Ethan Zisson[1], Joshua Wang[1], Joshua Greenburg[1], Judith Berman[3], Christina Cuomo[2], Richard Bennett[1]. 1) Molecular Microbiology & Immunology, Brown University, Providence, RI 02912; 2) Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142; 3) Tel Aviv University, 418 Britannia Building, Ramat Aviv 69978, Israel.

Candida albicans is a commensal organism of the human gastrointestinal tract and a prevalent opportunistic pathogen. Here, we begin to address the spectrum of natural diversity present in this species by analysis of a diverse collection of 21 clinical isolates. These isolates were recovered from bloodstream and mucosal infections, as well as from commensal niches. Isolates were examined for a variety of *in vitro* properties including growth rate, filamentation, biofilm formation, and drug resistance. Strains were also evaluated for virulence in the *Galleria* insect model of infection. In parallel with phenotypic analyses, the diploid genomes of the 21 strains were sequenced. Genetic variation between isolates was primarily due to differences in single nucleotide polymorphisms (SNPs), loss of heterozygosity (LOH) tracts, and aneuploid chromosomes. Overall genome heterozygosity varied from 48% to 89%, and a positive correlation was observed between strains with higher levels of heterozygosity and faster growth rates *in vitro*. This indicates that heterozygosity is positively associated with enhanced *in vitro* fitness. Furthermore, more than one-third of *C. albicans* strains were aneuploid, with 7 of the 21 strains being trisomic for one or more chromosomes. Aneuploidy was associated with a fitness defect, with aneuploid strains growing more slowly, on average, than strains with a euploid complement of chromosomes. Finally, we addressed the virulence defect of one clinical isolate and show that a SNP mutation in the *EFG1* transcription factor is associated with this phenotype. Loss of this factor decreased virulence but improved fitness in a commensal model of growth, indicating a natural mutation that alters the balance between commensal and pathogenic lifestyles.

Sexual development in euascomycetes: The STRIPAK complex is required for signaling during fruiting body formation. Ulrich Kück, Minou Nowrousian, Ines Teichert, Sandra Bloemendal, Anna Beier, Steffen Nordzieke, Eva Steffens. General and Molecular Botany, Ruhr University, Bochum, Germany.

The homothallic euascomycete *Sordaria macrospora* is a model system for studying sexual fruiting body (perithecia) formation. Previously, we generated a library of mutants with a developmental block in fruiting body formation, making *S. macrospora* an excellent system for studying cellular differentiation at the molecular level [1]. Analysis of a number of developmental mutants led to the identification of subunits of the conserved eukaryotic striatin-interacting phosphatase and kinase (STRIPAK) protein complex [2]. In particular, we identified PP2A scaffolding subunit PP2AA, catalytic subunit PP2Ac1, the striatin homolog PRO11, STRIP1/2 homolog PRO22, MOB3 homolog SmMOB3, and SLMAP homolog PRO45. Super-resolution structured-illumination microscopy (SIM) further established that PRO45 localizes to the nuclear envelope and mitochondria. SIM also showed that localization to the nuclear envelope required STRIPAK subunits PRO11 and PRO22. Using STRIPAK subunits as baits, we generated by mass spectrometry a high-confidence STRIPAK protein-protein interaction network, which was further verified by yeast-two hybrid and co-immunoprecipitation data. Our results indicate that the STRIPAK complex interacts with components of the cell wall integrity pathway, including its recently discovered scaffold protein PRO40 [3]. From the sum of our data we conclude that a crosstalk between different signaling complexes is crucial for the coordinated regulation of cellular differentiation during fruiting body formation.

[1] Teichert I, et al. (2014) Adv Genet 87: 202-246
[2] Bloemendal S, et al. (2012) Mol Microbiol 84: 310-323

[3] Teichert I, et al. (2014) PLoS Genet 10: e1004582.

γ-Tubulin: a Multifunctional Cell Organizer. Berl R. Oakley. Molecular Biosciences, University of Kansas, Lawrence, KS.

Our finding that deletion of the *mipA* (γ-tubulin) gene in *Aspergillus nidulans* results in a virtually complete blockage of mitotic apparatus assembly contributed to a generally accepted model that complexes containing γ-tubulin serve as templates that nucleate microtubule assembly in all eukaryotes. Our lab, however, has created a number of conditional *mipA* alleles in which robust mitotic spindle formation occurs under conditions in which growth is inhibited. Microscopic examination revealed that the mutant alleles cause a variety of mitotic and cell cycle defects, suggesting that γ-tubulin has important functions for growth in addition to microtubule nucleation. We have now examined this possibility through intragenic complementation analysis of *mipA* alleles and our data indicate that γ-tubulin has at least three important functions. To explore these functions, we created fluorescent protein fusions of a large number of proteins involved in mitosis and cell cycle regulation and observed the localization patterns of these proteins by time-lapse microscopy in wild-type and *mipA* mutant strains. These observations have revealed that the activity of a key cell cycle regulatory complex, the anaphase promoting complex/cyclosome (APC/C), is regulated in part by the exclusion of the key activator protein Cdc20 from interphase nuclei and entry of Cdc20 into the nucleoplasm when the nuclear pore complexes partially disassemble at mitotic onset. Likewise the spindle assembly checkpoint (SAC) is regulated by the physical separation of components prior to mitotic onset after which they come to co-localize at kinetochores. Analysis of a cold-sensitive *mipA* allele reveals that γ-tubulin plays a key role in inactivation of the APC/C coupled with the activator protein Cdh1 at G_1/S and that this correlates with the failure of Cdh1 to leave the spindle-pole body (SPB). The same allele causes nuclear autonomous mislocalization of the SAC proteins Bub1/R1 and Mps1, thereby abolishing the SAC in affected nuclei. Another allele inhibits binding of cytoplasmic dynein to the SPB at a temperature restrictive for growth. These data indicate that γ-tubulin plays an important role in the localization of mitotic and cell cycle regulatory proteins and, concomitantly, in the regulation of the cell cycle.

Regulation of microtubule-based transport. Samara Reck-Peterson[1], Anthony Roberts[1], Ekaterina Toropova[2], Nathan Derr[1], Brian Goodman[1], Sirui Zou[1], Andres Leschziner[2]. 1) Cell Biology, Harvard Medical School, Boston, MA; 2) Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Microtubule-based intracellular transport is a fundamental process in all eukaryotic cells, required for cell division, cell growth, and cell differentiation. We address how transport works on multiple scales, ranging from the biophysics of the motors, to their regulation and function in cells using both *S. cerevisiae* and *A. nidulans*. Cytoskeletal molecular motors move unidirectionally along their tracks. This poses multiple problems, which I will discuss: 1) How do they get to the start of their track? To determine how the microtubule-based motor dynein gets to the start of its track (the microtubule plus end) we reconstituted this process *in vitro*, showing that dynein is transported by a kinesin that wins a tug-of-war with dynein via the recruitment of processivity factors. 2) After arriving to the start of their track, how do they stay there until they have acquired cargo? A candidate for retaining dynein at microtubule plus ends is a ubiquitous regulator called Lis1. We showed that Lis1 anchors dynein to microtubules. It does so by binding directly to the dynein motor domain, resulting in uncoupling cycles of ATP hydrolysis from track binding. 3) How do cargos bound by motors of opposite polarity (both dynein and kinesin) undergo net unidirectional motility? Most cellular cargos are moved by groups of motors. We developed methods to construct 3D artificial cargos using DNA origami, to program the number, type, and spacing of motors, allowing us to dissect the mechanism of bi-directional microtubule-based transport. Collectively, our experiments so far with dyneins and kinesins linked to the same cargo suggest that unidirectional motility requires regulation.

Long-distance endosome trafficking drives fungal effector production during plant infection. Gero Steinberg. Sch Biosci, Univ Exeter, Exeter, United Kingdom.

To cause plant disease, pathogenic fungi can secrete effector proteins into plant cells to suppress plant immunity and facilitate fungal infection. Most fungal pathogens infect plants using very long strand-like cells, called hyphae, which secrete effectors from their tips into host tissue. How fungi undergo long-distance cell signalling to regulate effector production during infection is not known. Here, I summarize recently published data showing that long-distance retrograde motility of early endosomes (EEs) is necessary to trigger transcription of effector-encoding genes during plant infection by the pathogenic fungus *Ustilago maydi*s. This motor-dependent retrograde EE motility is controls effector production and secretion during host cell invasion. It involves the mitogen-activated kinase Crk1, which travels on EEs and participates in control of effector production. Fungal pathogens therefore undergo endosome-mediated long range-signalling to orchestrate host invasion. These findings add to recent discovery that EEs distribute the machinery for protein translation. Thus, 15 years after the discovery of motile endosomes in fungi, the biological role of their motility is beginning to reveal.

PLENARY SESSION ABSTRACTS

Friday, March 20 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session III: Interactions
Chair: Corby Kistler

Cell-cell communication and fusion in *Neurospora crassa*. Andre Fleissner. Institut fuer Genetik, TU Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany.
While cell fusion is essential for the development of most eukaryotic organisms, the molecular mechanisms underlying this process are only poorly understood. In recent years, *Neurospora crassa* has been adopted as an experimental model to study cell fusion and related cell-cell signaling processes.

Germinating conidia of this fungus sense each other and fuse. As a result, a germling network is formed, which further develops into the mycelial colony. Genetic analysis combined with live-cell imaging revealed an unusual mode of communication during these spore interactions. The two fusion partners appear to switch between signal sending and receiving thereby establishing a kind of "cell-cell dialog". This interaction involves the alternating recruitment of the MAK-2 MAP kinase module and the SO protein to the plasma membrane. Our further analysis revealed that the composition of the plasma membrane is critical for a proper cell-cell interaction. Mutants accumulating specific ergosterol precursors are deficient in germling fusion, particularly in the processes after cell-cell contact. While the membrane recruitment of MAK-2 is mostly unaffected in these strains, SO strongly mislocalizes. SO interacts with another MAP kinase module, the MAK-1 pathway. In wild type, MAK-1 is recruited to the fusion point after cell-cell contact, but fails to accumulate in the sterol mutants. Inhibition of MAK-1 in a chemical genetics approach reproduces the phenotype of the sterol mutants. Together these data indicate that specific minor changes in the ergosterol molecule structure can exert major effects on specific signal transduction pathways.

In addition, we recently found that the SO protein interacts with factors of a Calcium-dependent membrane repair system, which is activated in aberrant fusion events and in response to drug-induced cell lysis.

In conclusion, our data suggest the presence of an intricate signaling network controlling and linking cell-cell communication, plasma membrane fusion and repair.

New insights into effector research in the sebacinoid root endophyte *Piriformospora indica*. Alga Zuccaro[1,2]. 1) Organismic Interactions, Max Planck Institute Marburg, Marburg, Germany; 2) University of Cologne, Botanical Institute, Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.
Root endophytism and mycorrhizal associations are complex, derived traits in fungi that shape plant physiology. Sebacinales (Hymenomycetes, Basidiomycetes) display highly diverse interactions with plants. While basal lineages are root endophytes loosely colonizing root cells and/or have saprotrophic abilities, derived clades are biotrophs forming mycorrhizal associations, sometimes uncultivable. Sebacinales thus document the transition from saprotrophy to endophytism and to mycorrhizal nutrition within one family. Genomic traits associated with this transition are keys to the understanding of fungal root symbioses and will be discussed together with recent advances in effector research in the sebacinoid root endophyte *Piriformospora indica*. A special focus will be given on functional insights into the molecular mechanisms of symbioses with the monocot host plant barley and the dicot model plant *Arabidopsis thaliana*.

Evolution of virulence in the vascular wilt pathogen *Verticillium dahliae*. L. Faino, M.F. Seidl, D.E. Cook, X. Shi-Kunne, J.C. Boshoven, H. Rovenich, M. van Damme, J. Li, E. Rojas Padilla, Y. Song, D.J. Valkenburg, G.C. van den Berg, B.P.H.J. Thomma. Lab Phytopathology, Wageningen Univ, Wageningen, Netherlands.
Fungi cause severe crop losses and threaten food security worldwide. The soil-borne fungal pathogen *Verticillium dahliae* causes vascular wilt disease on hundreds of plant species, and disease control is challenging because resistance in plants is relatively rare. Moreover, *V. dahliae* has a flexible genome allowing it to escape host immunity and maintain aggressiveness. So far, knowledge on mechanisms governing this genomic flexibility remains limited.

Through comparative population genomics we have started to unravel mechanisms to establish the genomic diversity that is essential for adaptive genome co-evolution during the continued arms race with host plants. To this end, two *V. dahliae* genomes were assembled from telomere-to-telomere using long-read sequencing technology and optical mapping, and compared these to the genomes of other *Verticillium* spp., revealing a pre-speciation genome duplication event. Comparative genomics using the two finished *V. dahliae* genomes furthermore revealed recent segmental duplications that established lineage-specific regions. Interestingly, these regions are enriched for in planta-expressed effector genes encoding secreted proteins that enable host colonization, and thus contribute to the evolution of virulence. Our evidence suggests that error-prone homology-dependent DNA repair has caused genomic rearrangements, leading to extensive structural variations. Re-sequencing of additional strains showed that independent losses of genetic material favored the escape of host recognition and, likely, host specificity. We propose that evolution of *V. dahliae* is linked to segmental genome duplications mediated by improperly repaired DNA breaks.

In addition to genome evolution, we also study the role of epigenetic modifications on virulence of *V. dahliae* and the biological functions of effector proteins. Collectively, these research lines provide insight in mechanisms that make this fungus such a successful broad host range pathogen.

Microsporidia infection in *C. elegans* and other hosts. Emily Troemel. Division of Biological Sciences, Univ California, San Diego, La Jolla, CA.

Microsporidia comprise a phylum of over 1400 species of obligate intracellular parasites related to fungi. These parasites can infect virtually all animal hosts, and are responsible for widespread disease in humans as well as agriculturally relevant hosts such as fish and insects. Several years ago we identified a new genus in microsporidia called *Nematocida*, which contains natural pathogens of *Caenorhabditis* nematodes and provide model systems for studying microsporidian pathogenesis. We have sequenced several *Nematocida* genomes, and compared them with other microsporidia genomes, to identify evolutionary events of gene loss, acquisition, and modification. In particular, we found that all microsporidia lost the tumor suppressor gene Retinoblastoma, which could accelerate the parasite cell cycle. We found that microsporidia acquired transporters that could import nucleosides to fuel rapid growth. Also, microsporidian hexokinases gained secretion signal sequences, and in a functional assay these were sufficient to export proteins out of the cell; thus hexokinase may be targeted into the host cell to reprogram it toward biosynthesis. Similar molecular changes appear during formation of cancer cells and may be evolutionary strategies adopted independently by microsporidia to proliferate rapidly within host cells. In addition, analysis of genome polymorphisms revealed evidence for a sexual cycle that may provide genetic diversity to alleviate problems caused by clonal growth. Altogether these events may explain the emergence and success of these diverse intracellular parasites.

In addition to our microsporidian genome studies, we have been analyzing the *C. elegans* host response to microsporidia infection. We have described host resistance strategies as well as host trafficking pathways exploited by microsporidia to facilitate their spread and propagation. We are translating these findings into microsporidia infections of human cells to learn more about how these ubiquitous pathogens cause disease.

Fungus-insect interactions: genomics and molecular biology. Xiao Hu, Guohua Xiao, Peng Zheng, Chengshu Wang. Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, CAS, Shanghai 200032, China.

There are about one thousand fungal species capable of killing insects, most of which are ascomycetes. The species like *Metarhizium* spp. and *Beauveria bassiana* has been developed as environmentally friendly biocontrol agents against different insect pests. The species like the caterpillar fungus *Ophiocordyceps sinensis* and *Cordyceps militaris* etc., however, have been used as traditional Medicines for hundreds of years in Eastern countries. Genome analyses revealed that the insect pathogens have a strikingly larger proportion of genes encoding secreted proteases and chitinases than other sequenced fungi. Phylogeny analysis confirmed that fungal entomopathogenicity is polyphyletic, so similar expansions of insect cuticle degrading enzymes reflect a convergent evolution unique for fungus-insect interactions. In terms of the evolution of fungal host specificity, our genome survey of different *Metarhizium* species with varied host range indicated the presence of transitional species with intermediate host ranges and that the specialist species with a narrow host range diverged first and then the transitional species followed by the generalists in association with the evolution of insect hosts. Our molecular biology studies indicated that the insect fungi like *M. robertsii* evolved with divergent adhesins to mediate spore adherence to insect and plant surfaces, a mammalian perilipin-like protein to regulate the appressorium turgor pressure for insect cuticle penetration and a collagen-like protein to camouflage cell wall components for evading host immunity. The genomes of insect pathogenic fungi also encode an array of gene clusters for biosynthesis of secondary metabolites. Along with the elucidation of the biosynthetic mechanisms of the cyclodepsipeptide destruxins in *M. robertsii* and dibenzoquinone oosporein in *B. bassiana*, we showed that these metabolites could be deployed by the fungi to inhibit host innate immune responses and thereby facilitate fungal propagation within insect body cavity. The results of our studies advanced the understanding of genomics and molecular studies of fungus-host interactions.

Saturday, March 21 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session IV: Signals
Chair: Kathy Borkovich

Investigating the cell cycle control of plant infection by the rice blast fungus *Magnaporthe oryzae*. Nick Talbot, Yogesh Gupta, Miriam Oses-Ruiz, Lauren Ryder, Wasin Sakulkoo, Yasin Dagdas, Michael Kershaw, Darren Soanes, George Littlejohn, Magdalena Urdiroz. School of Biosciences, University of Exeter, Exeter, United Kingdom.

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, focused as mechanical force to breach the rice cuticle and facilitate entry into plant tissue. We observed that a single round of mitosis occurs prior to appressorium morphogenesis and precedes autophagic cell death of the three-celled conidium, which is necessary for plant infection. An S-phase checkpoint is necessary for initiation of appressorium development and maturation of the appressorium requires the G2-M transition.Furthermore, penetration peg emergence from the appressorium requires S-phase to have occurred in the appressorium nucleus. Re-polarisation of the appressorium requires a hetero-oligomeric septin GTPase complex for re-organisation of a toroidal F-actin network at the base of the appressorium. This allows the host cuticle to be breached and leads to invasion of epidermal cells by biotrophic

invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. The appressorium pore is also the site of polarised exocytosis during plant infection and the octameric exocyst complex localises to the pore in a septin-dependent manner and is essential for cytoskeletal regulation. Both cell cycle and pressure-mediated checkpoints appear necessary for initiation of septin activation and re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion. Once tissue is invaded the fungus undergoes differential expression of a large repertoire of effector proteins destined either for the apoplastic space, bounded by the plant plasmalemma, or directed instead into plant cells. How this delivery is achieved in not known, but involves a specialised structure known as the biotrophic interfacial complex (BIC), a plant membrane-rich body where effectors accumulate.

Intercellular communication and morphogenesis in the human fungal pathogen *Cryptococcus neoformans*. Xiaorong Lin. Biology, Texas A&M University, TAMU-3258, TX.

As non-mobile eukaryotic microbes mostly live in a community rather than in a planktonic state in the environment and in the host, it is important to know how they communicate and coordinate their community behaviors. Our recent findings in *Cryptococcus*, a major human fungal pathogen, indicate that secreted fungal adhesion proteins that are essential in creating the extracellular matrix for the formation of biofilms, are also critical in coordinating community behaviors (e.g. biofilm formation, morphogenesis, and sexual reproduction). This is achieved *via* the dual functions of these molecules as adhesins as well as signaling molecules. This concept of matrix-initiated signaling in intercellular communication in microbial communities is analogous to matrix-regulated cell differentiation and tissue patterning in higher eukaryotes.

RNA silencing in *Mucor*: small RNAs in development and pathogenesis. Rosa M. Ruiz-Vazquez. Dept Genetics and Microbiology, Univ Murcia, Murcia, Spain.

Mucor circinelloides is an opportunistic human pathogen evolutionary distant from other fungal model organisms. It is a causal agent for the rare but lethal infection mucormycosis, an emerging infectious disease recognized as a prevalent fungal infection in patients with impaired immunity, although recently it has been described in some otherwise healthy individuals. Besides its emerging pathogenicity, *M. circinelloides* also outstands for being one of the first fungi in which endogenous small RNAs (esRNAs) with putative regulatory functions have been identified. Different classes of esRNAs derived from exons (ex-siRNAs) have been identified, which differ in the components of the silencing machinery required for their biogenesis. These ex-siRNAs regulate the expression of the protein coding genes from which they are produced. In fact, whole-genome transcriptional analysis reveals that different combinations of RNAi proteins regulate the expression of specific gene clusters, which are up- or down-regulated in the corresponding RNAi mutants. The RNAi mutants are affected in general developmental processes such as growth and sporulation, and show differential response to environmental signals, such as nutritional, oxidative and heat stresses. Some of these processes are regulated by the canonical RNAi pathway, whereas others, such as sexual interaction, are regulated by a *dicer*-independent non-canonical RNAi pathway. We have also investigated the role of the RNAi machinery in pathogenesis by using distinct host-pathogen interaction models to identify genes and ex-siRNAs differentially expressed during infection. Results in zebrafish and inmunocompromised mice using virulent and avirulent strains of *M. circinelloides*, as well as RNAi mutants, suggest a role for the RNAi machinery in pathogenesis.

This work was funded by the Spanish MICINN (BFU2009-07220) and MINECO (BFU2012-32246) co-financed by FEDER.

Interplay between self and nonself recognition mechanisms regulate chemotropic interactions and cell fusion. N. Louise Glass, Wilfried Jonkers, Jens Heller, Abigail Leeder, Gabriel Rosenfield, Monika Fischer, David Kowbel. Dept Plant & Microbial Biol, Univ California, Berkeley, CA.

In filamentous fungi, fusion between genetically identical cells is associated with the colony establishment and hyphal network formation. In *Neurospora crassa*, both germling fusion (via conidial anastomosis tubes; CATs) and hyphal fusion in a mature colony require a conserved MAP kinase cascade: a MAPKKK (NRC-1), a MAPKK (MEK-2), a MAPK (MAK-2) and a Ste12-like transcription factor, PP-1. Chemotropic interactions are associated with oscillation of MAK-2, MEK-2 and NRC-1 in germlings and fusion hyphae. This MAPK cascade is highly conserved, but a scaffold protein analogous to *S. cerevisiae* Ste5 has not been identified in filamentous ascomycete fungi. By utilizing phosphoproteomics and co-immunoprecipitation, we identified a scaffold protein (HAM-5) for the MAK-2/MEK-2/NRC-1 complex. HAM-5 co-oscillated with NRC-1, MEK-2 and MAK-2 with identical dynamics during chemotropic interactions. In a Δ*mak-2* strain, HAM-5 localized to puncta that did not oscillate, suggesting that MAK-2 kinase activity regulates HAM-5 function/localization. The identification of the HAM-5 scaffold protein will link the activation of this MAPK cascade to upstream factors and proteins involved in fungal communication. To determine whether communication/chemotropism between genetically different germlings was similar or different to communication/chemotropism between genetically identical germlings, we used a population genomics approach with 112 wild isolates of *N. crassa*. Chemotropic interactions between genetically different germlings was associated with three communication groups (CGs). By bulked segregant analysis, we identified two highly polymorphic genes (determinant of communication: *doc-1* and *doc*-2) that show evidence of balancing selection and are associated with CG phenotype. During chemotropic interactions, DOC-1 co-oscillated and physically interacted with the HAM-5/MAK-2/MEK-2/NRC-1 complex. These data indicate that self and nonself interactions during germling fusion are linked and that an interplay between these two systems is important in the regulation of chemotropic interactions and cell fusion.

PLENARY SESSION ABSTRACTS

Analysis of clock-regulated genes in *Neurospora* reveals widespread posttranscriptional control of metabolic potential. Jennifer M. Hurley[1], Arko Dasgupta[1], Jillian Emerson[1], Xiaoying Zhou[1], Carol Ringelberg[1], Nicole Knabe[1], Anna Lipzen[2], Erika Lindquist[2], Christopher Daum[2], Kerrie Barry[2], Igor Grigoriev[2], Kristina Smith[3], James Galagan[4], Deborah Bell-Pedersen[5], Michael Freitag[3], Chao Chang[1], Jennifer Loros[1,6], Jay Dunlap[1]. 1) Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) US Department of Energy Joint Genome Institute, Walnut Creek, CA 94598; 3) Department of Biochemistry and Biophysics, Center of Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331; 4) Departments of Biomedical Engineering and Microbiology, Boston University, Boston, MA 02215; 5) Department of Biology, Texas A&M University, College Station, TX 77843; 6) Department of Biochemistry, Geisel School of Medicine, Hanover, NH 03755.

Neurospora crassa has been for decades a principal model for filamentous fungal genetics and physiology as well as for understanding the mechanism of circadian clocks. Eukaryotic fungal and animal clocks comprise transcription-translation–based feedback loops that control rhythmic transcription of a substantial fraction of these transcriptomes, yielding the changes in protein abundance that mediate circadian regulation of physiology and metabolism: Understanding circadian control of gene expression is key to understanding eukaryotic, including fungal, physiology. Indeed, the isolation of clock-controlled genes (*ccg*s) was pioneered in *Neurospora* where circadian output begins with binding of the core circadian transcription factor WCC to a subset of *ccg* promoters, including those of many transcription factors. High temporal resolution (2-h) sampling over 48 h using RNA sequencing (RNA-Seq) identified circadianly expressed genes in *Neurospora*, revealing that from ~10% to as much 40% of the transcriptome can be expressed under circadian control. Functional classifications of these genes revealed strong enrichment in pathways involving metabolism, protein synthesis, and stress responses; in broad terms, daytime metabolic potential favors catabolism, energy production, and precursor assembly, whereas night activities favor biosynthesis of cellular components and growth. Discriminative regular expression motif elicitation (DREME) identified key promoter motifs highly correlated with the temporal regulation of *ccg*s. Correlations between *ccg* abundance from RNA-Seq, the degree of *ccg*-promoter activation as reported by *ccg*-promoter–luciferase fusions, and binding of WCC as measured by ChIP-Seq, are not strong. Therefore, although circadian activation is critical to *ccg* rhythmicity, posttranscriptional regulation plays a major role in determining rhythmicity at the mRNA level.

Wednesday, March 18 3:00 PM–6:00 PM
Merrill Hall

Multicellular Development/Crosstalk between Morphogenetic and Developmental Pathways in Filamentous Fungi
Co-chairs: Minou Nowrousian and Oier Etxebeste

The interplay of a GPI-anchored protein and the STRIPAK complex regulate fruiting-body development in the filamentous ascomycete *Sordaria macrospora*. Stefan Frey[1], Yasmine Lahmann[1], Stefanie Poeggeler[1,2]. 1) Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August University Göttingen, Germany; 2) Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University Göttingen, Germany.

The striatin interacting phosphatase and kinase (STRIPAK) complex, which is composed of striatin, protein phosphatase PP2A and kinases, is required for fruiting-body development and cell fusion in the filamentous ascomycete *Sordaria macrospora* [1]. Here, we report on the interplay of the glycosylphosphatidylinositol (GPI)-anchored protein SmGPI1 with the kinase activator SmMOB3, a core component of human and fungal STRIPAK complexes. SmGPI1 is conserved among filamentous ascomycetes and was first identified in a yeast two-hybrid screen using SmMOB3 as bait. The physical interaction of SmMOB3 and SmGPI1 was verified by co-Immunoprecipitation. *In vivo* localization and differential centrifugation revealed that SmGPI1 is predominantly secreted and attached to the cell wall but is also associated with mitochondria and appears to be a dual-targeted protein. Deletion of *Smgpi1* led to an increased number of fruiting bodies that were normally shaped but reduced in size. In addition, *Smmob3* and *Smgpi1* genetically interacted. In the sterile ΔSmmob3 background deletion of *Smgpi1* restores fertility, vegetative growth as well as hyphal-fusion defects. The suppression effect was specific for the ΔSmmob3 mutant since deletion of *Smgpi1* in other STRIPAK mutants does not restore fertility.

[1] Bloemendal S, Bernhards Y, Bartho K, Dettmann A, Voigt O, Teichert I, Seiler S, Wolters DA, Pöggeler S, Kück U (2012) A homologue of the human STRIPAK complex controls sexual development in fungi. Mol Microbiol 84:310-323.

Identification of NoxD/Pro41 as the homologue of the p22 NADPH oxidase subunit in fungi. Isabelle Lacaze[1], Hervé Lalucque[1], Ulrike Siegmund[2], Philippe Silar[1], Sylvain Brun[1]. 1) LIED-UMR 8236, Univ Paris-Diderot, Paris, France; 2) Institut für Biologie und Biotechnologie der Pflanzen, Westfälische Wilhelms Universität, Schlossplatz 8, D-48143 Münster, Germany.

NADPH oxidases (Nox) are membrane complexes that produce O_2^- by transferring electrons from cytosolic NADPH to oxygen. In Eukaryotes, Nox are involved in major processes like cell proliferation, differentiation and defense. In mammals, O_2^- production by Nox2 relies on the activation of the membrane flavocytochrome b_{558} composed of the catalytic subunit Nox2/gp91 and of p22 by a cytosolic complex composed of p67, p47, p40 and the small GTPase Rac. In fungi, although the composition of the activating complex (p67/NoxR, Rac, Cdc24 and Bem1) is well conserved, the apparent lack of a homologue of *p22* in genomes questioned how the flavocytochrome forms at membranes. Three Nox isoforms are present in the ascomycetes model *Podospora anserina*: PaNox1, PaNox2 and PaNox3. PaNox1 is involved in fruiting body formation, anastomosis, defense, appressorium-like development and Crippled Growth. Remarkably, all these processes are impaired in the *IDC509* mutant strain as in *IDC343*, the *PaNox1* mutant strain. We have identified *Pa_1_7250* as the gene mutated in the *IDC509* strain. By careful phylogenetic and functional analyses, we show that *Pa_1_7250*, which is homologous *to SmPro41* of *Sordaria macrospora*, is the orthologue of mammalian *p22*. We therefore named it *NoxD* for *Nox Docking protein*. While the physical interaction between NoxD and Nox1 was demonstrated in the plant pathogen *Botrytis cinerea* by Ulrike Siegmund and collaborators in Paul Tudzynski's lab, our cytological analyses of functional tagged versions of PaNox1, PaNoxD and PaNoxR show that they co-localize in the endoplasmic reticulum and/or in the vacuolar system suggesting the assembly of an active Nox complex.

Diverse roles for the non-receptor guanine nucleotide exchange factor RIC8. Katherine Borkovich, Patrick Schacht, Asharie Campbell, Alexander Michkov, Shouqiang Ouyang, Arit Ghosh. Plant Pathology & Microbiology, University of California, Riverside, CA.

Heterotrimeric G proteins consist of α, β and γ subunits. We have previously characterized three Gα subunits (GNA-1, GNA-2 and GNA-3), one Gβ (GNB-1) and one Gγ (GNG-1) subunit in *Neurospora crassa*. Previous work in our laboratory has shown that the cytosolic RIC8 protein functions as a guanine nucleotide exchange factor (GEF) for the GNA-1 and GNA-3 Gα subunits, and is also required for maintenance of these two proteins, via a post-transcriptional mechanism. Results from *ric8* structure-function studies and ribosomal profiling experiments have revealed insights into the RIC8 residues that are required for GEF activity, as well as details of the post-transcriptional mechanism leading to low Gα levels in *N. crassa*.

A 'developmental hourglass' in mushroom-forming fungi. X. Cheng, Y.Y. Lee, M.C. Wong, H.S. Kwan. School of Life Sciences, The Chinese University of Hong Kong, New Territory, Hong Kong.

The 'developmental hourglass' concept suggests that vertebrates are most similar to one another during mid-embryogenesis, and this highly conserved stage is illustrated by the 'waist' of the hourglass representing a low probability of evolutionary change. Recent molecular surveys on both animals and plants have shown that the genes expressed at the waist stage are more ancient and more conserved in their expression. The existence of such a developmental hourglass has not been explored in fungus, a eukaryotic kingdom that evolved with multicellularity. In this study, we analyzed two series of whole-genome expression data on mushroom development – a microarray assay on *Coprinopsis cinerea* and an RNA-seq profiling on *Lentinula edodes*. We found that both mushrooms display a molecular hourglass pattern over their developmental lifecycles. The 'young fruiting body' (YFB) is the stage that expresses the evolutionarily oldest (lowest

transcriptome age index, TAI) transcriptome. To put the developmental pattern into functional context of mushroom development, we categorized expressed genes into Eukaryotic Orthologous Groups (KOG). We found that the expression of genes in 'information storage and processing' reached a maximum at YFB and decreased later at the MFB, while genes in 'metabolism' was the lowest at YFB. The synchronic existence of a molecular 'hourglass' across species reveals a common strategy used by eukaryotes to incorporate evolutionary innovations.

The role oxidative stress and RNP granules have in spore survival. Steven Gorsich, Michelle Steidemann, Alyssa Litwiller, Megan Postema. Department of Biology, Central Michigan University, Mt Pleasant, MI.

The meiotic generation of gametes (e.g. spores, pollen grains, oocytes, spermatocytes) is an essential function for sexually reproducing eukaryotes. The ability of plants, animals, and fungi to produce sexual gametes and store them for extended periods of times is essential for the survival of these organisms. For instance, plant pollen grains can be viable for centuries and human oocytes and baleen whales can have viable oocytes for as many as 50 and 90 years, respectively. Interestingly, less is known about the survivorship of fungal spores. Experimental evidence shows that some fungal spores last at least 6 years, while anecdotal evidence suggests that fungal spores can last over 25 years. Though these different types of gametes are less viable as they age, the fact that they remain genetically stable for decades is impressive. Using *Saccharomyces cerevisiae* we are investigating the cellular mechanisms that allow spores to survive for extended periods of time. More specifically, we are interested in the role genetics, environmental stress, and ribonucleoprotein (RNP) granules have on spore development. Previously, we have shown that specific gene mutations (e.g. *dnm1*) lead to defects in spore survival and fitness. In this study, we will present data that describe: 1) how oxidative stress (reactive oxygen species, ROS) effects spore viability and mitochondrial morphology, 2) how the overexpression of *ZWF1* protects yeast against ROS damage during spore development, 3) how the overexpression of *ZWF1* lessens the effects of the *dnm1/dnm1* mutant, and 4) our characterizations of the RNP granules, P-bodies and stress granules, during spore development. These data suggest that *S. cerevisiae* require the ability to protect their developing spores against oxidative stress and the ability to form RNP granules during spore development.

Patterns of cellular morphogenesis during conidiophore development in *Aspergillus nidulans*. Steven Harris. Center Plant Sci Innovation, Univ Nebraska, Lincoln, NE.

Spores are the primary propagules by which fungi disseminate. Thus, they represent an attractive target for the control of fungal colonization and infection. Although conserved signaling pathways and transcriptional networks that regulate sporulation have been identified, relatively little is known about how the morphogenetic events that underlie the production of spores are integrated with these pathways. My lab uses the model filamentous fungus *Aspergillus nidulans* to characterize mechanisms that regulate fungal morphogenesis. Here, I will describe our initial characterization of a regulatory module that mediates the production of spores in a repeated pattern by phialides. A key component of this module, Axl2, is a predicted cell surface protein that appears to control the transition from acropetal to basopetal growth in phialides. The absence of Axl2 disrupts septin recruitment and results in the formation of shortened chains consisting of only one or two spores. Because expression of Axl2 is controlled by the transcription factors BrlA and AbaA, transcriptional profiling was used to identify additional components of the module. These include the conserved protein kinase NdrA as well as a novel protein (PhiB) that is predicted to bind chitin. The absence of either of these proteins also leads to the production of shortened spore chains. In addition, like Axl2, NdrA only localizes to phialide-spore junctions. Preliminary analysis of double mutants suggests that Axl2 and NdrA define parallel pathways for the regulation of phialide morphogenesis.

Thioredoxins are essential for appressorium formation, conidiation, and circadian rhythm in *Magnaporthe oryzae*. C Jiang[1,2], SJ Zhang[2], JR Xu[1,2]. 1) Purdue University, West lafayette, IN; 2) Northwestern A&F University, Yangling, China.

In the rice blast fungus *Magnaporthe oryzae*, the Mst11-Mst7-Pmk1 MAP kinase (MAPK) pathway is essential for appressorium formation and infectious growth. Because the redox status can affect the dimerization of MAPK kinases and activation of downstream MAP kinases, in this study we isolated and characterized the two thioredoxin genes, *TRX1* and *TRX2*, in *M. oryzae*. *TRX2* had a higher expression level and was found to be the predominant thioredoxin gene. Whereas the *trx1* mutants had no detectable phenotypes except a minor reduction in conidiation, the *trx2* mutant rarely produced conidia and was down-regulated in the expression of *COM1*, *HTF1*, and *CON7* transcription factor genes. It was also significantly reduced in growth rate, aerial hyphal growth, and appressorium formation, and only caused rare, non-typical lesions on rice seedlings. However, the *trx1 trx2* double mutant was blocked in conidiation and conidiophore development and it was non-pathogenic in infection assays with hyphal fragments or culture blocks. Germ tubes and hyphal tips of the *trx2* and *trx1 trx2* mutants were defective in appressorium formation and failed to penetrate and grow invasively in plant cells. In the *trx2* and *trx1 trx2* mutants, the phosphorylation level of Pmk1 but not Mps1, was significantly reduced. Western blot analysis indicated that deletion of *TRX2* affected proper folding or dimerization of the Mst7 MEK kinase that functions upstream from Pmk1. Site-specific mutagenesis indicated that Cys305 of Mst7 is important for its function. Therefore, *TRX2* may affect appressorium formation and invasive growth via Pmk1. In addition, we found that *TRX2* also plays a role in responses to oxidative stress, accumulation of intracellular ROS, and circadian rhythm. Taken together, our data showed that the thioredoxin genes play important roles in intracellular ROS signaling, conidiogenesis, hyphal growth, and pathogenesis in *M. oryzae*.

Light regulation controls asexual and sexual development in *Aspergillus nidulans*. Reinhard Fischer. Dept of Microbiology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

Light perception has been a crucial step in evolution, because it is not only used as primary energy source of photosynthetic organisms, but also for orientation in the environment. Only a few different chromophores have evolved as photoreceptors, some of which are retinal-, flavin- or tetrapyrrol-based. Those chromophores are bound to proteins and undergo e.g. conformational changes upon light perception. The chromophore changes hence cause conformational changes of the protein, which in turn change their activity. Light responses may be

fast, but can also involve slow developmental changes of the entire organism. The latter response normally requires the differential regulation of hundreds or thousands of genes. In the case of filamentous fungi, many physiological and developmental processes are controlled by light. For instance *Aspergillus nidulans* senses red and blue light, which control the balance between asexual and sexual reproduction but also secondary metabolite formation. We have discovered phytochrome as one of the photoreceptors in this fungus and thus demonstrated that this chromophore is conserved from plants to lower eukaryotes. Transcriptional analyses revealed that hundreds of genes are differentially expressed upon illumination. Taking two of the genes, the mechanism of light regulation has been studied. We found that phytochrome along with a flavin-based blue-light receptor controls gene induction. In a novel approach we are trying to identify new components of the light-sensing system using mutagenesis. “Blind” mutants were isolated, and their characterization is under way. The aim is to identify the corresponding mutations by whole-genome sequencing.

Wednesday, March 18 3:00 PM–6:00 PM
Chapel

Epigenetics, Chromatin, and Genome Defense
Co-chairs: Isabelle Fudal and Zachary Lewis

Whole genome HiC analysis of interactions between chromosomal regions in wildtype Neurospora and in mutants defective in heterochromatin machinery. Jonathan Galazka[1], Andrew Klocko[2], Eric Selker[2], Michael Freitag[1]. 1) Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Eukaryotic genomes are organized into chromatin domains with non-random three-dimensional arrangements that presumably result from interactions of nucleic acid and protein factors within the physical constraints of the nucleus. It is of obvious interest to determine interactions between various chromosomal regions defined by these nuclear constraints, as well as to identify important factors that limit the interactions. We used chromosome conformation capture (3C) followed by high-throughput Illumina sequencing (HiC) to improve our understanding of the organization of the Neurospora genome and to test the involvement of known components of heterochromatin machinery in nuclear organization. We were most interested in regions classified as "constitutive" and "facultative" heterochromatin, defined by methylation of histone H3 on lysine 9 (H3K9me3) or methylation of lysine 27 (H3K27me3), respectively. H3K9me3 results from action of the DIM-5 methyltransferase, whereas H3K27me3 results from the action of the SET-7 methyltransferase of the PRC2 complex. We carried out HiC on chromatin from wildtype, and from *dim-5* and *set-7* mutants defective in the deposition of these histone "marks". In addition, we characterized the *hpo* mutant, which is deficient in HP1, an important heterochromatin protein known to bind H3K9me3. Our data for the genome configuration of wild type nuclei revealed intra- and inter-chromosomal associations between heterochromatic domains, with the strongest interactions among the centromeres and telomeres. We found that neither loss of HP1, nor loss of the H3K9me3 and H3K27me3 marks drastically disrupted chromatin organization. Nevertheless, we did observe changes in the chromatin organization in these strains. Thus, our datasets not only define critical domains important for higher-order genome patterning, but establish a baseline to which future mutants will be compared.

Heterochromatin components are requried for the normal genome integirty. Takahiko Sasaki, Zackary Lewis. Department of Microbiology, University of Georgia, Athens, GA. 1000 Cedar Street, Biological science building Room 824, Athens, GA 30602.

In response to genotoxic stress, ATR and ATM kinase phosphorylate H2A in fungi and H2AX in animals on a C-terminal serine. The resulting modified histone, called γH2A, recruits chromatin-binding proteins that stabilize stalled replication forks or promote DNA double strand break repair. To identify genomic loci that might be prone to replication fork stalling or DNA breakage in *Neurospora crassa*, we performed chromatin immunoprecipitation (ChIP) of γH2A followed by next-generation sequencing (ChIP-seq). γH2A-containing nucleosomes are enriched in *Neurospora* heterochromatin domains. These domains are comprised of A:T-rich repetitive DNA sequences associated with histone H3 methylated at lysine-9, the H3K9me-binding protein Heterochromatin Protein-1 (HP1), and DNA cytosine methylation. H3K9 methylation, catalyzed by DIM-5, is required for normal γH2A localization. In contrast, γH2A is not required for H3K9 methylation or DNA methylation. Normal γH2A localization also depends on HP1 and a histone deacetylase, HDA-1, but is independent of the DNA methyltransferase, DIM-2. γH2A is globally induced in Δ*dim-5* mutants under normal growth conditions, suggesting that the DNA damage response is activated in these mutants in the absence of exogenous DNA damage. Together, these data suggest that heterochromatin formation is essential for normal DNA replication or repair.

Identification of Neurospora Shelterin. Miki Uesaka, Ayumi Yokoyama, Shinji Honda. Life Science Unit, University of Fukui, Eiheiji, Fukui, Japan.

A telomere-specific protein complex, Shelterin, caps and protects chromosome ends against inappropriate DNA damage, telomeric fusion and telomere length in eukaryotes. However, in any filamentous fungi, the corresponding Shelterin has not been identified. Here we show Neurospora Shelterin which is composed of at least five proteins. The two components, POT-1 and RAP-1, are conserved from yeasts to mammals whereas the others are only conserved in Ascomycota. By Chromatin Immunoprecipitation (ChIP) assays, we confirmed that one of Neurospora Shelterin components is specifically localized to telomeres. Fluorescence microscopic analyses revealed that all the components of Neurospora Shelterin are co-localized to 2~4 foci within nuclei. The telomeric foci are mostly associated with nuclear envelope and heterochromatin but not a single centromeric spot. Furthermore, the localization is not dependent of H3K9 methylation that directs heterochromatin and DNA methylation.

Epigenetic and transcriptional control in coordination of fungal development and secondary metabolism. Gerhard Braus. Microbiol/Gen, Molec Microbiol, Georg-August Univ, Goettingen, Germany.

Differentiation and secondary metabolism are correlated processes in fungi that respond to various abiotic or biotic external triggers. The velvet family of regulatory proteins plays a key role in coordinating secondary metabolism and differentiation processes as asexual conidia formation or the formation of resting structures. Such overwintering structures are associated with a specific secondary metabolism presumably for defense against other organisms of the habitat. The velvet domain family shares a protein domain that is present in most parts of the fungal kingdom from chytrids to basidiomycetes. Velvet domain proteins interact with several epigenetic methyltransferases which affect fungal secondary metabolism. The COP9 signalosome (CSN) protein complex is located genetically upstream of the velvet proteins and is conserved from fungi to mammals. CSN is required for fungal development as a multicellular organism but in contrast to mammals, CSN is not essential for fungal viability. CSN physically interacts with DenA which has a different impact on fungal development. The last years have revealed a complex genetic network how the coordination between secondary metabolism and development is controlled on different hierarchical levels which will be discussed.

Heterochromatic marks play an important role in regulating the symbiotic interaction between *Epichloë festucae* and *Lolium perenne* and symbiosis-specific secondary metabolite gene expression. Tetsuya Chujo[1,2], Yonathan Lukito[1], Murray Cox[1], Barry Scott[1]. 1) Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Genes for the synthesis of ergot alkaloid (*eas*) and indole-diterpene (*ltm*) bioprotective metabolites are organised as clusters in the sub-telomeric regions of the genome of *Epichloë festucae*, a fungal symbiont of *Lolium perenne*. These genes are all highly expressed *in planta* but not expressed in axenic culture. To explain these observations we propose that these alkaloid gene loci have a repressive chromatin structure in culture and that chromatin remodeling is important for activation of these gene clusters *in planta*. To test this hypothesis we perfomed ChIP-qPCR, and found that levels of histone H3 lysine 9 and lysine 27 trimethylation (H3K9me3/H3K27me3) were reduced at these gene loci *in planta* compared to axenic culture[1]. Deletion of *E. festucae* genes encoding the H3K9- (ClrD) or H3K27- (EzhB) methyltransferases led to derepression of *ltm* and *eas* gene expression under non-symbiotic culture conditions. These changes in gene expression were matched by corresponding reductions in H3K9me3 and H3K27me3 marks. Similarly, deletion of *E. festucae hepA*, which encodes a homolog of heterochromatin protein-1, led to derepression of these gene loci. Each of the three mutants had distinct culture and symbiotic interaction phenotypes. While the culture morphology and radial growth of *ΔezhB* was like wild-type, both *ΔhepA* and *ΔclrD* had reduced radial growth, with the latter very severe. When *L. perenne* plants were inoculated with each of these three mutants, *ΔclrD* failed to infect, *ΔezhB* induced a late onset hypertillering phenotype whereas *ΔhepA* induced stunting of tiller growth and premature senescence of the host. To gain further insight into possible mechanism(s) underlying some of these changes we have compared the transcriptomes of wild-type and *ΔhepA* symbiota using RNAseq. In summary, these results highlight the importance of the epigenetic state in controlling fungal-plant symbiosis and alkaloid biosynthesis. 1) Chujo & Scott (2014). Mol Microbiol 92: 413-434.

Epigenetic hotspots are genomic islands for putative effector-encoding genes in *Zymoseptoria tritici*. Jessica L. Soyer[1,2,3], Jonathan Grandaubert[2,3], Klaas Schotanus[2,3], Janine Haueisen[2,3], Eva H. Stukenbrock[2,3]. 1) INRA UR 1290 BIOGER - CPP Avenue Lucien Brétignières, BP 01, 78850 Thiverval-Grignon, France; 2) Max-Planck-Institut for Evolutionary biology, August-Thienemann-Str. 2, 24306 Plön, Germany; 3) Christian-Albrechts University of Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany.

The Dothideomycete *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*) is a pathogen of wheat (*Triticum aestivum*) and has a hemibiotrophic life style suggesting a fine-tuned regulation of gene expression. The genome of *Z. tritici* comprises 21 chromosomes including up to eight conditionally dispensable chromosomes (CDCs) containing a considerably higher proportion of transposable elements (TEs) than core chromosomes (Goodwin *et al*., 2011). Evidence is accumulating that genes involved in host-pathogen interaction, such as effector-genes, can be located in TE-rich, heterochromatic genomic regions. In *Z. tritici*, while CDCs are TE-rich, they harbor few effector-genes. We however hypothesized that effector-genes are not randomly located on core chromosomes. In order to investigate their location with respect to the chromatin structure, a genome-wide histone map was established using ChIP-sequencing and correlated with TE, effector-gene location and transcriptomic data. As expected, CDCs are mainly heterochromatic while gene-rich core chromosomes are mainly euchromatic, which is consistent with the low expression of genes located on CDCs compared to core chromosomes. TE-rich regions of the core chromosomes are also enriched in histone modifications typical of heterochromatic domains, suggesting that an epigenetic control of expression of the genes located in these regions could occur. We define these regions of the core chromosomes as “epigenetic hotspots”. Analysis of the location of effector-genes in relation to epigenetic hotspots highlighted that these genes are significantly associated with heterochromatic domains. We further investigated effect of this location on the regulation of expression of these pathogenicity-related encoding genes during two stages of wheat infection. We conclude that TE-rich regions of core chromosomes define genomic environments for putative effector-encoding genes and that histone modifications may control their expression.

Small RNAs - the secret agents for fungal attacks. Hailing Jin[1], Arne Weiberg[1], Ming Wang[1], Feng-Mao Lin[2], Hongwei Zhao[1], Isgouhi Kaloshian[3], Hsien-Da Huang[2]. 1) Department of Plant Pathology & Microbiology, University of California, Riverside, CA., USA; 2) National Chiao Tung University, Taiwan; 3) Department of Nematology, University of California, Riverside, CA, USA.

Most fungal genomes encode components of RNAi machinery, including Dicer-like proteins and Argonautes (AGOs). The role of fungal small RNAs in genome defense, heterochromatin formation, and gene regulation has been demonstrated. However, it was not known whether fungal small RNAs or RNAi are directly involved in pathogenicity.

Botrytis cinerea is an aggressive fungal pathogen that infects more than 200 plant species. Genome-wide small RNA profiling from *B. cinerea*-infected Arabidopsis and tomato has identified a group of small RNAs from *B. cinerea* that can potentially target important

regulatory genes in plant hosts. Genetic and biochemical studies have demonstrated that some *B. cinerea* small RNAs (Bc-sRNAs) can selectively silence host immunity genes by hijacking host RNAi machinery. These Bc-sRNAs are loaded into host AGO proteins to silence host genes involved in defense. Deviated from the conventional pathogen protein effectors that suppress host immunity in plants and animals, we demonstrate that a fungal pathogen transfers "virulent" sRNA effectors into host cells to achieve infection. The implications of this finding may extend beyond grey mold disease or plant fungal diseases in general.

Investigating the RNA molecules of meiotic silencing by unpaired DNA (MSUD). Dilini A. Samarajeewa[1], Nicholas A. Rhoades[1], Hua Xiao[2], Kevin A. Edwards[1], Patrick K.T. Shiu[2], Thomas M. Hammond[1]. 1) School of Biological Sciences, Illinois State University, Normal, Illinois, 61790; 2) Division of Biological Sciences, University of Missouri, Columbia, Missouri, 65211.

In *Neurospora crassa*, meiotic silencing by unpaired DNA (MSUD) is a process that detects and silences unpaired DNA between homologous chromosomes during sexual development. It is believed that MSUD works through an RNA interference-related pathway that begins with the production of aberrant RNAs (aRNAs). However, these aRNAs have yet to be identified. Here, we present results from experiments designed to identify these theoretical molecules. Additionally, we present results from our analysis of a novel MSUD protein. This protein has RNA binding domains and it could be involved in transporting MSUD-related RNA molecules, such as aRNAs, to their proper destination in the meiotic cell.

Wednesday, March 18 3:00 PM–6:00 PM
Fred Farr Forum

Fungus-animal Interactions
Co-chairs: Robert Cramer and Stephanie Diezmann

***Candida albicans* commensalism in the mammalian host.** Suzanne Noble. Microbiology & Immunology; Medicine, UCSF School of Medicine, San Francisco, CA.

Among an estimated 5,000,000 fungal species, *Candida albicans* is the dominant commensal of the human gastrointestinal tract, as well as the most common invasive pathogen. We previously reported that exposure of *C. albicans* yeasts to the mammalian GI tract triggers a morphological switch between oval "white" cells, which are virulent in disseminated infections, and cigar-shaped "GUT" cells, which exhibit enhanced commensal fitness in a murine GI infection model. The white-to-GUT switch requires the Wor1 transcription factor, which also promotes sexual switching by rare *C. albicans* strains that have lost an allele of the Mating Type-Like Locus, *MTL*. Unlike the sexually competent "opaque" cell type, however, GUT cells are incapable of mating and lack other functional hallmarks of opaque cells whereas, unlike GUT cells, opaque cells are attenuated for commensalism. We hypothesize that *C. albicans* responds to specific signals from the mammalian GI tract to activate the white-to-GUT switch, thereby triggering a metabolic shift and other changes that are adaptive in this environment. To better understand the switch, we have developed an *in vitro* assay that elicits reversible, *WOR1*-dependent cell elongation of wild-type *MTL***a**/α cells. Using this tool, we have begun to dissect the host signals and fungal signaling pathways that trigger commensal switching behavior. Broadly speaking, the GUT cell type switch illuminates how a single organism can inhabit radically different host environments and transition between commensalism and invasive tissue pathogenesis.

The heat shock response governed by Hsp90 and Hsf1 is necessary for cell survival and virulence in the pathogenic fungus *Candida albicans*. Michelle Leach[1,2], Rhys Farrer[3], Koon Ho Wong[4], Christina Cuomo[3], Al Brown[2], Leah Cowen[1]. 1) Molecular Genetics Dept, University of Toronto, Toronto, Canada; 2) University of Aberdeen, IMS, Aberdeen, UK; 3) Broad Institute, Cambridge, MA, USA; 4) Faculty of Health Sciences, University of Macau, Macau.

Temperature is a ubiquitous environmental variable, adaptation to which is necessary for survival in all organisms. Upon exposure to a sub-lethal heat shock in yeast, normal metabolic functions become repressed and the heat shock transcription factor Hsf1 is activated, inducing heat shock proteins (HSPs). *Candida albicans*, the most prevalent human fungal pathogen, is an opportunistic pathogen that has evolved as a harmless commensal of healthy individuals. Even though *C. albicans* occupies thermally buffered niches, it has retained the classic heat shock response, activating Hsf1 during slow thermal transitions such as increases in temperature suffered by febrile patients. The molecular chaperone Hsp90 interacts with and down-regulates Hsf1 in *C. albicans*, modulating short-term activation of the heat shock response. To obtain a global picture of the heat shock response we have performed both RNA-seq and ChIP-seq in the absence and presence of heat shock to determine which genes Hsf1 binds and regulates. As expected, Hsf1 binds to and regulates heat shock proteins necessary for cell survival upon a heat shock. However, a subset of genes required for virulence was also upregulated and bound by Hsf1. These genes are not required for survival at high temperatures, with mutants lacking these genes displaying no growth defect upon heat shock. Our data suggest that Hsf1 is not only essential for regulating genes necessary for cell survival upon heat shock, but also genes required for virulence. Indeed, cells that have received a sub-lethal heat shock are more virulent in a *Galleria mellonella* model of infection compared to cells grown at 30°C. Finally, we show that depletion of *HSP90* drastically changes the signature of the heat shock response, blocking upregulation of many of these virulence genes. Therefore, Hsf1 and Hsp90 act in concert in the pathogen to combat the host response of fever by upregulating genes necessary for cell survival and continued infection.

Iron sources and siderophore production in *Paracoccidioides* spp. CELIA SOARES. Molecular Biology Laboratory, Institute of Biological Sciences, Federal University of Goias, Goiania, Goias, Brazil.

Iron sources and siderophore production in *Paracoccidioides* spp. Iron is essential for the proliferation of fungal pathogens during

infection. The availability of iron is limited due to its association with host proteins. Host iron sources used by *Paracoccidioides* spp. were investigated. *Paracoccidioides* spp. present hemolytic activity and have the ability to internalize a protoporphyrin ring. A hemoglobin receptor ortholog, Rbt5, was identified as a surface GPI-anchored protein that recognized hemin, protoporphyrin and hemoglobin in vitro. Antisense RNA technology and *Agrobacterium tumefaciens*-mediated transformation were used to generate mitotically stable Pbrbt5 mutants. The knockdown strain had a lower survival inside macrophages and in mouse spleen when compared with the parental strain, which suggested that Rbt5 could act as a virulence factor. In summary, our data indicate that *Paracoccidioides* spp. can use hemoglobin as an iron source most likely through receptor-mediated pathways that might be relevant for pathogenic mechanisms. Additionally, pathogens may present specific mechanisms for iron uptake, which include the production and secretion of siderophores, low-molecular weight ferric iron-specific chelators. Quantitative real time PCR demonstrated that genes involved in siderophore biosynthesis and transport are induced under iron starvation. Biochemical assays demonstrated that *Paracoccidioides* species produce and secret hydroxamate-type siderophores under iron deprivation. Reversed-phase HPLC and mass spectrometry analysis of culture supernatants revealed that the fungus secretes coprogen B. Ferricrocin and ferrichrome C were detected in *Paracoccidioides* as the intracellular produced siderophores. Cross-feeding experiments demonstrated that siderophores secreted by *Paracoccidioides* restored the growth of an *Aspergillus nidulans* mutant unable to grow in standard growth media unless siderophores are supplied. Together, these data denote that synthesis and utilization of siderophores are mechanisms used by *Paracoccidioides* to surpass iron limitation. As iron paucity is found within the host, iron uptake molecules production may be related to fungus pathogenicity.

Manipulation of macrophage biology by the intracellular fungal pathogen *Histoplasma capsulatum*. Bevin English, Young Nam Lee, Dervla Isaac, Charlotte Berkes, Anita Sil. Howard Hughes Medical Institute, Dept. of Microbiology and Immunology, UCSF, San Francisco, CA.

Histoplasma capsulatum (Hc) is a primary fungal pathogen of humans and other mammals. As an intracellular pathogen, Hc is able to subvert the immune function of naïve macrophages and replicate within the phagosome, eventually causing macrophage lysis. However, the mechanism by which Hc causes host-cell death is unknown. Macrophage lysis is dependent upon the secreted protein Cbp1 (calcium binding protein 1, ref 1); we show that *cbp1* mutant yeast are able to grow to high levels within macrophages, but these host cells do not lyse, indicating that high intracellular fungal burden is not sufficient to trigger host-cell death. Because Cbp1 has no known protein domains and only a few orthologs which are relatively unstudied, we undertook two exploratory approaches to begin to elucidate the mechanism by which Cbp1 mediates the interaction between Hc and its host. The first approach is a comprehensive alanine scanning mutagenesis of Cbp1, which enabled us to identify a group of acidic residues at the N terminus that are necessary for macrophage lysis. We are currently assessing the mutant library for other Cbp1 properties, such as calcium binding, which will enable us to either link or uncouple these properties to the ability to trigger host-cell death. The second exploratory approach was transcriptional analysis of infected macrophages, which led to the identification of a set of host genes that are induced during Hc infection in a Cbp1-dependent manner. Several of these host genes are involved in ER stress and apoptosis, including *Tribbles3* (*TRB3*). Here we show that macrophages deficient for *TRB3* are resistant to Cbp1-mediated lysis. Similarly, macrophages lacking both Bax and Bak, key components of the apoptotic pathway, are also resistant to lysis during Hc infection. These data suggest that Cbp1 induces apoptosis in the host cell, and we are currently investigating host factors that interact with Cbp1 to cause macrophage death.

Function and regulation of the Candida glabrata Pdr1 transcription factor. Sanjoy Paul[1], Thomas Bair[2], Hayes McDonald[3], W. Scott Moye-Rowley[1]. 1) Dept Molec Physiology/Biophy, Univ Iowa, Iowa City, IA; 2) Iowa Institute of Human Genetics, Univ Iowa, Iowa City, IA; 3) Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN.

Candida glabrata is an emerging pathogen found in up to 25% of candidemias. One of the major complications associated with *C. glabrata* infections is its facile acquisition of resistance to the azole drugs, the major clinical antifungal agent. Experiments from several labs have established that a key transcription factor called Pdr1 mediates transcriptional induction of a suite of genes that include ATP-binding cassette transporter proteins that ultimately produce azole resistance. Pdr1 is induced upon exposure to azole drugs and can be genetically activated by substitution mutations or in response to loss of the mitochondrial genome. While homologues of Pdr1 have been extensively studied in *Saccharomyces cerevisiae*, less is known about the function of the *C. glabrata* Pdr1. We have carried out two analyses aimed at determining unique aspects of the role of Pdr1 in this pathogen. First, chromatin immunoprecipitation coupled with high throughput sequencing (ChIP-seq) analysis was used to map the Pdr1 binding sites across the *C. glabrata* genome. While similarities were seen in the range of target promoters when compared to *S. cerevisiae*, important differences were also discovered. Second, a tandem affinity purification (TAP)-tagged form of *PDR1* was prepared and used to isolate this transcription from cells with or without a mitochondrial genome. Mass spectrometric analysis of purified protein indicated that factors involved in control of ubiquitination and protein folding were co-purified with TAP-Pdr1. Progress in understanding the role of these interactions in regulation of Pdr1 will be presented.

Characterization of Myosins in *Aspergillus fumigatus* Growth and Pathogenesis. Hilary Renshaw[1], Praveen R. Juvvadi[2], José M. Vargas-Muñiz[1], Amber D. Richards[2], William J. Steinbach[1,2]. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Pediatrics, Duke University Medical Center, Durham, NC.

Aspergillus fumigatus is the etiological agent of invasive aspergillosis, a leading cause of death in immunocompromised patients. Invasion of host tissue by this fungus is facilitated by polarized hyphal growth and septation. Myosins are a group of motor proteins known to be involved in hyphal growth, morphology and septation in fungi. However, the role of myosins in the growth and virulence of a human pathogen has never been explored. In this study, we investigated *A. fumigatus* myosins belonging to three different classes: class I (MyoA), class II (MyoB), and class V (MyoE). We generated two myosin single deletion strains (Δ*myoB* and Δ*myoE*) and a double deletant (Δ*myoB* Δ*myoE*). Attempts to delete the *myoA* gene were unsuccessful, suggesting its essential nature. While the Δ*myoB* strain showed aberrant

septation it was also hypersensitivity to anti-cell wall agents revealing its role in maintaining cell wall integrity. Deletion of *myoE* resulted in reduced hyphal extension and hyperbranching, indicating its role in preserving hyphal polarization and/or suppressing new growth foci. In addition, the Δ*myoE* strain displayed hyperseptation albeit with normal septa, suggesting that MyoE is necessary for regular septation. In contrast to the Δ*myoE* strain, the Δ*myoB* Δ*myoE* strain showed broader hyphae with reduced hyphal extension and absence of septation, indicating that both MyoB and MyoE are integral for septum formation. Both Δ*myoB* and Δ*myoE* strains were hypovirulent in a screening *Galleria mellonella* model of invasive aspergillosis; however, surprisingly, only the Δ*myoB* strain displayed decreased virulence in a neutropenic murine model of infection. Taken together, these data demonstrate the crucial but distinct roles that myosins play in maintaining proper hyphal morphology and for pathogenesis of *A. fumigatus*.

An extracellularly-produced peptide acts intracellularly to program fungal virulence. C. Homer[1], D. Summers[1], A. Goranov[1], S. Clarke[2], D. Wiesner[3], D. Tofaletti[4], J. Moresco[5], I. Caradonna[1], S. Petnic[1], J. Yates[5], J. Perfect[4], K. Nielsen[3], C. Craik[2], H. Madhani[1]. 1) Dept. of Biochemistry and Biophysics, UCSF , San Francisco, CA; 2) Dept. of Pharmaceutical Chemistry UCSF, San Francisco, CA; 3) 3Deparment of Microbiology Univ. of Minnesota Minneapolis, MN; 4) Dept. of Medicine Duke University Durham, North Carolina, USA; 5) Scripps Research Institute La Jolla, California, USA.

We report a peptide-based cell-to-cell signaling system required for virulence in the fungal pathogen *Cryptococcus neoformans*. *QSP1* encodes the precursor of a C-terminally-encoded 11 amino acid secreted peptide. We find that *qsp1*Δ mutants are significantly attenuated in a murine inhalation model, with a defect in pulmonary fungal burden, but no obvious difference in the pulmonary inflammatory response. In contrast, *qsp1*Δ cells do not show defects in a rabbit CNS infection model, suggesting a niche- or infection stage-specific role. Mutant cells proliferate normally *in vitro* under a variety of starvation and stress conditions, but exhibit a rough colony morphology, suggestive of surface changes. Indeed, the mutant displays slightly increased levels of polysaccharide capsule, a modest decrease in melanization, less organized cell wall ultrastructure, and increased sensitivity to the cell wall probe SDS. Qsp1 also impacts the pattern of extracellular protease activity. The ability of the synthetic Qsp1 peptide to complement the colony morphology phenotype of *qsp1*Δ cells is sensitive to most amino acid changes and truncations indicating a strong connection between Qsp1 structure and function. Biogenesis of Qsp1 requires an extracellular serine protease that liberates the mature peptide from its precursor. Qsp1 sensing does not require any known GPCR or two-component receptor, but instead requires a predicted oligopeptide transporter. These findings suggest that import of Qsp1 into cells is required for its action. Indeed, we observe that intracellular expression of the mature Qsp1 peptide reverts colony morphology phenotype of the null mutant. Thus, Qsp1 is an extracellularly-matured peptide that promotes density sensing and virulence and that possesses at least one intracellular site of action.

Identification of conserved and novel features of the alkaline response pathway in the fungal pathogen *Cryptococcus neoformans*. Kyla Selvig[1], Teresa O'Meara[2], Naureen Huda[1], Shannon Esher[1], J. Andrew Alspaugh[1]. 1) Department of Medicine, Duke University, Durham, NC; 2) Department of Molecular Genetics, University of Toronto, Toronto, CANADA.

The Rim/Pal pathway is a conserved fungal signaling pathway responsible for sensing and responding to alkaline extracellular pH. This pathway was first identified and characterized in members of the ascomycete phylum. Here, we analyze the Rim/Pal pathway in the opportunistic fungal pathogen, *Cryptococcus neoformans*, a member of the basidiomycete phylum. We found that like in ascomycete fungi, the *C. neoformans* Rim101 transcription factor is proteolytically activated in response to alkaline pH. Rim101 activation requires the ESCRT pathway along with three conserved Rim pathway components, Rim23, Rim20, and Rim13. Interestingly, *C. neoformans* and related basidiomycete fungi lack clear orthologs for the most upstream components of the pathway, which are responsible for sensing extracellular pH and activating the pathway. To identify these unknown Rim signaling components, we performed a random mutagenesis screen specifically designed to identify activators of the *C. neoformans* Rim pathway. From this screen, we identified a novel protein, which we named *RRA1* (Required for Rim101 Activation 1). *RRA1* appears to be specifically conserved in basidiomycete fungi, indicating that *RRA1* is likely a previously unidentified component of the basidiomycete Rim pathway. Supporting this hypothesis, we found that disrupting the *RRA1* ortholog in *Cryptococcus gattii* produced mutant phenotypes identical to a *C. gattii rim101Δ* mutant. *RRA1* is predicted to encode a membrane protein that is structurally similar to the Rim21/PalH pH membrane receptor in the ascomycete Rim pathway. However unlike the Rim21/PalH pH receptor, Rra1 does not appear to be localized on the plasma membrane or on intracellular vesicles, but is primarily localized to the endoplasmic reticulum. Future experiments will determine the specific role of Rra1 in Rim pathway activation, and whether the ER is the true site of Rra1 function. These studies have significantly advanced our understanding of the mechanisms of environmental sensing and adaptation in *C. neoformans* and other basidiomycetes.

Wednesday, March 18 3:00 PM–6:00 PM
Kiln

Secondary Metabolism
Co-chairs: Robert Proctor and Masayuki Machida

Secondary metabolism in *Fusarium fujikuroi*: genome mining and hierarchical regulatory networks. Bettina Tudzynski[1], Lena Studt[1], Slavica Janevska[1], Eva-Maria Niehaus[1], Andreas Pfannmüller[1], Sarah Rösler[1], Caroline Michielse[1], Birgit Arndt[2], Hans-Ulrich Humpf[2]. 1) Inst Biology and Biotechnology, University Munster, Munster, Germany; 2) Institut of Food Chemistry, University Munster, Munster, Germany.

The recent comprehensive analysis of the high-quality genome sequence of the gibberellin (GA)-producing fungus *F. fujikuroi* revealed the presence of about 50 mostly unknown secondary metabolite (SM) gene clusters. The expression of most of these clusters depends on

nitrogen availability and pH. Some of these gene clusters could be linked to particular products. However, most of them are still cryptic and often silent. In order to activate those cryptic gene clusters we use different approaches in our group: 1) Overexpression of pathway-specific transcription factors, 2) Deletion and and overexpression of global regulators, (AreA, AreB, Velvet, Lae1, Sge1), and 3) Deletion and overexpression of genes involved in histone modifications.

By overexpressing the pathway-specific transcription factors we were able to activate two cryptic *F. fujikuroi*-specific SM clusters (PKS19 and NRPS31) that are not present in any other sequenced fungal genome. Structure elucidation revealed a new family of polyketides named **fujikurins** and a new cyclic tetrapeptide, **apicidin F (APF)**, respectively. The APF gene cluster is an example for the complex and hierarchical regulation of secondary metabolism. Beside the APF-specific transcription factor APF2, the nitrogen regulator AreB and the global regulator Sge1 are essential for cluster gene expression, while AreA does not play any role. Overexpression of *APF2* led to significant up-regulation of the cluster genes. However, deletion of Sge1 in the OE:*APF2* background almost completely abolished the activating effect of APF2 suggesting that Sge1 acts at a higher regulation level, e.g. chromatin remodeling. Overexpression of Sge1 is sufficient to overcome repression of altogether 3 nitrogen-induced SM gene clusters (fusarin, fusaric acid and APF) underlining its important role as master regulator of secondary metabolism. Furthermore, deletion of histone-modifying genes also resulted in deregulation of several SM gene clusters.

The evolution of secondary metabolite degradation by specialization of gene clusters. Jason Slot[1], Antonis Rokas[2], George Greene[2,3], Emile Gluck Thaler[1]. 1) Plant Pathology, The Ohio State University, Columbus, OH; 2) Biological Sciences, Vanderbilt University, Nashville, TN; 3) Program in Genetics and Genomics, Duke University, Durham, NC.

The ability to neutralize or degrade host secondary metabolites is a key component of the metabolism of fungi that interact with plants. Many fungi possess metabolic adaptations that enable them to use specific plant defense compounds as a carbon source. Nutritional exploitation of plant defenses by fungi is important because it has been associated with increased virulence of fungal pathogens on a population level, and also because many of these metabolic pathways may be useful for degradation of pollutants that resemble defense compounds. Recently, we have identified gene clusters in Pezizomycotina, which putatively degrade phenolic plant defense compounds. These gene clusters are specialized adaptations of tyrosine catabolism with a spotty, ecological signature to their distribution, which has been influenced by horizontal gene transfer. Our analyses of remodeling of specialized clusters for phenolic compound degradation suggest that metabolic rewiring has occurred as an evolutionary response to specialization on alternative phenolic substrates. We present our investigation of putative stilbene degradation clusters in relation to diversification of plant secondary metabolism and fungal niche.

Fine scale evolution of fungal secondary metabolism. Kathryn Bushley[1], Stephen Rehner[2], Joseph Spatafora[3]. 1) Department of Plant Biology, University of Minnesota, Saint Paul, MN; 2) Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland 20705; 3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, United States of America.

Modular fungal secondary metabolite genes such as nonribosomal peptide synthetases and polyketide synthases, are among the most rapidly evolving of fungal genes. They respond to selective pressures in the environment, often allowing fungi to adapt to specific environments and hosts. Secondary metabolite gene clusters containing these core genes is also highly dynamic in terms of both gene composition, syntenic relationships among genes, and chromosomal locations. Thus, "evolutionary tinkering" at the levels of both genes and gene clusters can lead to novel chemical products. While the evolution of NRPS and PKS genes has been studied both broadly across the fungal kingdom and within specific groups and genera of fungi, patterns of evolution of these rapidly evolving genes are often difficult to infer across these larger taxonomic distances. Focusing on host and geographically diverse isolates of the cosmopolitan soil fungus and insect pathogen *Tolypocladium inflatum* and strains within the highly diverse *Beauveria bassiana* species complex, the fine-scale evolution of secondary metabolite genes and clusters is examine at the population level. The relative contribution of mechanisms such as transposition, selection, recombination, and gene conversion in driving the diversification or loss of secondary metabolites genes and clusters as well as the degree to which mechanisms are conserved across these two groups of fungi is explored.

Discovery of fungal secondary metabolic pathways from large-scale genomic and transcriptome information. M. Umemura[1], N. Nagano[2], H. Koike[1], K. Tamano[1], K. Abe[3], K. Shin-ya[4], K. Asai[2], M. Machida[1]. 1) Bioproduction Res. Inst., AIST, Sapporo, Japan; 2) Computational Biol. Res. Center, AIST, Tokyo, Japan; 3) Grad. Sch. Agricultural Sci., Tohoku Univ., Sendai, Japan; 4) Biomedical Res. Inst., AIST, Tokyo, Japan.

Fungi produce numerous secondary metabolites, many of which are bioactive and valuable for medicinal uses. Major biosynthetic pathways responsible for the synthesis of their main skeletal structures are polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs) and terpene cyclases, which are detectable in genome sequences owing to their sequence motifs. Other unknown types of secondary metabolite biosynthetic (SMB) pathways can even still exist, because it is just a fraction of the metabolites whose biosynthetic pathways are elucidated in filamentous fungi. Here, to explore genome information for SMB pathways, a motif-independent method, MIDDAS-M, is presented. The MIDDAS-M algorithm is based on the characteristics that SMB genes are clustered and cooperatively expressed on a fungal genome. It can detect novel types of SMB pathways using only gene-annotated genome information and transcriptome data. Thanks to this method, the fungal secondary metabolite, ustiloxin B, turned out to be produced by a ribosomal peptide synthetic (RiPS) pathway, which is the first report for filamentous fungi excluding *Amanita* mushroom. This RiPS pathway is quite unique and different from other known bacterial ones because its precursor protein contains 16-fold repeated core peptides that construct the cyclic portion of the compound. Based on the characteristics, other fungal RiPS pathways were searched and two corresponding novel RiPS compounds have been recently identified. In addition to RiPS, unknown but functionally characteristic gene clusters are sharply detected by MIDDAS-M, which might broaden the field of fungal secondary metabolites further.

CONCURRENT SESSION ABSTRACTS

Examining the evolution of the regulatory circuit controlling secondary metabolism and development in *Aspergillus*. Abigail Lind[1], Jennifer Wisecaver[2], Timothy Smith[3], Xuehan Feng[3], Ana Calvo[3], Antonis Rokas[1,2]. 1) Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Biological Sciences, Vanderbilt University, Nashville, TN; 3) Biological Sciences, Northern Illinois University, Dekalb, IL.

Filamentous fungi produce diverse secondary metabolites (SMs) essential to their ecology and adaptation. Although each SM is typically produced by only a handful of species, global SM production is governed by widely conserved transcriptional regulators in conjunction with other cellular processes, such as development. We examined the interplay between the taxonomic narrowness of SM distribution and the broad conservation of global regulation of SM and development in *Aspergillus*, a diverse fungal genus whose members produce well-known SMs. Evolutionary analysis of the 2,124 genes comprising the 262 SM pathways in four *Aspergillus* species showed that most SM pathways were species-specific, that the number of SM gene orthologs was significantly lower than that of orthologs in primary metabolism, and that the few conserved SM orthologs typically belonged to non-homologous SM pathways. RNA sequencing of two master transcriptional regulators of SM and development, *veA* and *mtfA*, showed that the effects of deletion of each gene, especially *veA*, on SM pathway regulation were similar in *A. fumigatus* and *A. nidulans*, even though the underlying genes and pathways regulated in each species differed. In contrast, examination of the role of these two regulators in development, where 94% of the underlying genes are conserved in both species showed that whereas the role of *veA* is conserved, *mtfA* regulates development in the homothallic *A. nidulans* but not in the heterothallic *A. fumigatus*. Thus, the regulation of these highly conserved developmental genes is divergent, whereas–despite minimal conservation of target genes and pathways–the global regulation of SM production is largely conserved. We suggest that the evolution of the transcriptional regulation of secondary metabolism in *Aspergillus* represents a novel type of regulatory circuit rewiring and hypothesize that it has been largely driven by the dramatic turnover of the target genes involved in the process.

A trichothecene biosynthetic enzyme complex and a potential mechanism for cellular trichothecene traffic in *Fusarium graminearum*. Karen Broz, Marike Boenisch, Burcu Yordem, H. Corby Kistler. USDA ARS Cereal Disease Lab and University of Minnesota, 1551 Lindig St., St. Paul, MN 55108.

The plant pathogenic fungus *Fusarium graminearum* produces the terpenoid mycotoxin deoxynivalenol (DON) and DON derivatives during infection of wheat as well as when induced in culture. Transcription factor Tri6p regulates expression of genes for enzymes in the mevalonate pathway (primary metabolism) as well as the DON biosynthetic pathway (secondary metabolism). Genes for both pathways display similar patterns of expression during infection, suggesting DON synthesis requires pathway coordination. When strains with fluorescently labeled enzymes are grown in medium conducive to DON induction, the mevalonate pathway enzyme HMG CoA reductase (Hmr1) co-localizes with the DON biosynthetic enzymes Tri1 and Tri4 in spherical membranous structures called "toxisomes". Strict co-localization of Hmr1p, Tri4p, and Tri1p suggest that the enzymes may be part of a multi-enzyme complex, a hypothesis supported by fluorescence resonance energy transfer (FRET) between fluorescently tagged proteins. To further characterize proteins of the toxisome, fluorescence-activated cell sorting (FACS) was used to enrich for Tri4p::RFP tagged toxisomes for proteomic analysis. Preliminary analysis of the FACS-enriched proteome revealed co-enrichment for additional enzymes involved in trichothecene and terpene synthesis. While toxisomes are the proposed site of DON biosynthesis, other subcellular compartments may mediate export of DON. GFP tagging of the trichothecene transporter Tri12p revealed localization to the plasma membrane as well as to vacuoles and small (1 μm) motile vesicles which interact with toxisomes. Motile Tri12 vesicles labeled with GFP fuse with the vacuole or plasma membrane, suggesting that vesicular transport of DON may play a role in cellular sequestration and export of the toxin. The t-SNARE protein directing subapical exocytosis (SSO1) may be involved in toxin export. Deletion mutants (Δsso1) are significantly reduced in the ability to accumulate DON and a DON derivative in toxin induced cultures. Our results suggest a role for vesicular trafficking and exocytosis in export of DON.

Clues to an evolutionary mystery: the genes for T-toxin, enabler of the devastating 1970 Southern Corn Leaf Blight epidemic, are present in ancestral species. Bradford Condon[1], Candace Elliott[2], Sung-Hwan Yun[3], Youhei Haruki[4], Motochiro Kodama[4], B. Gillian Turgeon[1]. 1) Department of Plant Pathology, Cornell University, Ithaca, NY; 2) School of Botany, The University of Melbourne, Parkville 3010 VIC, Australia; 3) Department of Medical Biotechnology, Soonchunhyang University, Asan, Chungnam 336-745, South Korea; 4) Laboratory of Plant Pathology, Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan.

The Southern Corn Leaf Blight epidemic of 1970 devastated fields of T-cytoplasm corn planted in monoculture throughout the eastern US. The epidemic was driven by race T, a previously unseen race of the fungal Dothideomycete pathogen, *Cochliobolus heterostrophus*. Race T produces T-toxin, a polyketide host selective toxin encoded by the genetically complex *Tox1* locus. Despite forty years of research, a definitive inventory of the genes at *Tox1* and their evolutionary origin have remained a mystery. Here we show that an Eurotiomycete species, *Penicillium raistrickii*, and two additional Dothideomycete species, *Corynespora cassiicola* and *Leptosphaeria maculans*, possess all of the known *Tox1* genes, at a single collinear genetic locus. The compact gene clusters in these species are in stark contrast to the genetically disjointed *C. heterostrophus* race T *Tox1* locus, and this finding suggests that the race T arrangement cannot be the ancestral state. Furthermore, the clusters facilitate the definition of the *Tox1* genetic boundaries. The *Tox1*-like gene discovery in other species, especially in an Eurotiomycete, offers an opportunity to tackle the *Tox1* evolutionary timeline for the first time, as the Eurotiomycete/Dothideomycete split is estimated to have occurred ~320-400 MYA. However, phylogenetic analyses of PKS-encoding genes are difficult because such genes have rapid evolution signatures and notoriously discontinuous distribution patterns. Thus, despite finding *Tox1*-like gene clusters in species ancestral to *C. heterostrophus*, we find it difficult to distinguish between a history of discontinuous evolution mediated by loss, gain, and recombination and a history of horizontal gene transfer.

Comparative genomics and gene cluster identification in 28 species of *Aspergillus* section *Nigri*. Tammi Vesth[1], Jane Nybo[1], Sebastian Theobald[1], Ellen K. Lyhne[1], Martin E. Kogle[1], Igor Grigoriev[3], Uffe H. Mortensen[1], Scott E. Baker[2], Mikael R. Andersen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Joint BioEnergy Institute, Emeryville, CA and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; 3) Joint Genome Institute, Walnut Creek, CA, USA.

The filamentous fungus *Aspergillus niger* and its close relatives in *Aspergillus* section *Nigri* are of broad interest to the scientific community including applied, medical and basic research. The fungi are prolific producers of native and heterologous proteins, organic acids (in particular citrate), and secondary metabolites (including bioactives and toxins such as ochratoxin A). Because of these abilities they represent a substantial economic interests in bioenergy applications. While 8 individual species from this group has been whole-genome sequenced, the genetic basis for these diverse phenotypes remains largely unidentified.

In this study, we have de novo sequenced the genomes of 20 additional species of the section *Nigri*, thus allowing the genome comparison of all members of this important section of fungal species. Here we present the results of this large-scale genomic analysis where we have examined the core genome of these 28 species and identified variations in the genetic makeup of individual species and groups of species. In particular, we have found genes unique to *Aspergillus* section *Nigri*, as well as genes which are only found in subgroups of the section. Our analysis here correlates these genes to the phenotypes of the fungi.

Furthermore, we have predicted secondary metabolite gene clusters in all 28 species. We present here an overview of these gene clusters and how they are shared and vary between species. We also correlate the presence of gene clusters to presence of known fungal metabolites.

Wednesday, March 18 3:00 PM–6:00 PM
Heather

Biodiversity of Fungi
Co-chairs: Linda Kohn and Georgianna May

Scaling fungal diversity from soil cores to continents. Kabir Peay[1], Jenny Talbot[2], John Taylor[3], Rytas Vilgalys[4], Thomas Bruns[2]. 1) Biology, Stanford University, Stanford, CA; 2) Biology, Boston University, Boston, CA; 3) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 4) Biology, Duke University, Durham, NC.

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. Understanding how macroecological processes shape fungal communities is in part hindered by the lack of datasets spanning large spatial-scales and the absence of distributional data for most fungi. To rectify this knowledge gap we used next generation sequencing of the internal transcribed spacer region of the nrRNA genes to survey soil fungi across North American pine forests, spanning a diverse range of climates from Florida to Alaska. At each site we took 26 soil samples arranged in a nested grid and stratified into organic and mineral soil horizons. At the present we have obtained sequence data from over 1000 individual soil samples. Using this spatially explicit sampling design we examine patterns of fungal diversity from very small to large spatial scales and across steep local environmental gradients. We also compare patterns of diversity across different fungal taxonomic and functional groups. Using this unprecedented dataset we can make inferences about the key drivers of fungal biodiversity and the scales at which they are most important.

Fungi as drivers of microbial community assembly: moving from patterns to molecular mechanisms. Benjamin Wolfe[1], Rachel Dutton[2]. 1) Biology, Tufts University, Medford, MA; 2) FAS Systems Biology, Harvard University, Cambridge, MA.

From an increasing number of high-throughput sequencing surveys, broad patterns of eukaryotic and prokaryotic microbial community diversity are beginning to emerge. However, our understanding of processes driving these patterns of diversity is still limited due to the complexities of experimentally manipulating most microbial communities. Using the fungal and bacterial communities from cheese rinds as a tractable model system, we are determining the processes and molecular mechanisms by which fungi can shape diversity of multi-species microbial communities. Across 137 different cheese rind communities, fungal diversity is positively correlated with bacterial diversity. When grown in pairwise co-culture experiments, bacteria are more responsive to the presence of fungi than fungi are to bacteria, suggesting that biotic interactions with fungi can be major drivers of community assembly. Using a combination of *in vitro* community reconstructions, RNA-seq, and comparative genomics, we're identifying the molecular mechanisms by which fungi structure bacterial communities. Compositional overlap with other microbial communities and conserved molecular mechanisms driving species interactions will allow us to translate our findings from cheese rinds to less tractable microbial communities.

Geographic and temporal structure of endophytic and endolichenic fungal communities of the boreal biome. Jana M. U'Ren[1], Francois Lutzoni[2], Jolanta Miadlikowska[2], Thomas Gleason[1], Ashton Leo[1], James T. Monacell[3], Kayla Arendt[1], Emilie Lefevre[2], Bernard Ball[2], Ko-Hsuan Chen[2], Georgiana May[4], Ignazio Carbone[3], A. Elizabeth Arnold[1,5]. 1) School of Plant Sciences, University of Arizona, Tucson, AZ; 2) Department of Biology, Duke University, Durham, NC 27708 USA; 3) Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695 USA; 4) Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN 55108 USA; 5) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721 USA.

Although species-rich in all terrestrial communities, endophytic and endolichenic fungi reach their greatest phylogenetic diversity in boreal forests -- earth's largest forest biome and the most threatened due to climate change. Previous studies have used single sampling events and culture-based methods to infer spatial-, geographic-, and host affiliations of these fungi. Here we utilize a collection of >16,000 cultures in conjunction with next-generation sequencing from the same host tissues to examine endophytic and endolichenic communities from sets of 20 plant and lichen species sampled (a) in multiple years at one boreal site and (b) in eight sampling sites distributed along the entire boreal belt. This study reveals previously unexplored temporal stability of fungal communities, with a particularly high stability in long-lived lichen thalli. Endolichenic fungi were consistently more abundant in culture than endophytic fungi isolated from co-occurring hosts, yet species diversity was similar among endophytic and endolichenic communities. We observed strong geographic structure in fungal communities, with most fungal OTUs occurring only at a single site. Ongoing work will highlight the complementarity of culture-based and culture-free methods, and examine the role of biotic and abiotic factors in structuring symbiotic fungal communities of the boreal biome at both regional and continental scales.

Evolution of specificity in the lichen-forming genus *Peltigera* and its cyanobacterial partner: consequences on speciation rate and geographical range. Jolanta Miadlikowska[1], Nicolas Magain[2], Bernard Goffinet[3], Emmanuel Sérusiaux[2], François Lutzoni[1]. 1) Department of Biology, Duke University, Durham, NC; 2) Evolution and Conservation Biology, University of Liège, Liège, Belgium; 3) Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT.

Variation in specificity among symbiotic partners is key to a comprehensive understanding of the evolution of symbiotic systems. This variation is expected to occur within species as well as within a broader inter-species phylogenetic framework. We assessed the level of specificity of lichen-forming *Peltigera* species of the section *Polydactylon* (mycobiont) and their cyanobacterial partner *Nostoc* (cyanobiont), by inferring the phylogeny of the mycobiont, based on five nuclear loci, and of their respective cyanobionts, using the *rbcLX* region. A total of 208 lichen thalli, representing ca. 40 putative *Peltigera* species, were sampled worldwide. We found a broad spectrum of specificity for both partners, ranging from strict specialists to generalists. However, mycobionts are usually more specialized than cyanobionts by associating mostly with one or a few *Nostoc* phylogroups, whereas cyanobionts associate frequently with several *Peltigera* species. A relatively recent colonization of a new geographic area (South America) seems correlated with a switch to a generalist pattern of association by the mycobiont, and an increased rate of diversification. Specialization of the mycobiont seems to be acquired through time, i.e., favored in areas where species have been established for long periods of time, and to be associated with lower mycobiont genetic diversity. Overall, geographic ranges of these *Peltigera* species increase with the number of phylogroups with which they can form successful symbioses. Therefore, the evolution of specificity seems to play a key role in defining the geographical range of mycobiont species and cyanobiont phylogroups, and in mediating shifts in diversification rates of these *Peltigera* species.

Priority effects during fungal community establishment in beech wood. Jennifer Hiscox[1], Melanie Savoury[1], Sarah Johnston[1], Carsten Müller[1], Björn Lindahl[2], Hilary Rogers[1], Lynne Boddy[1]. 1) Cardiff University, School of Biosciences, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX; 2) Swedish University of Agricultural Sciences, Dept. of Soil and Environment, Box 7014, SE-750 07 Uppsala, Sweden.

Fungal communities in decomposing wood change with time, often beginning with relatively ruderal species and those establishing from propagules latently present in functional sapwood. These are subsequently replaced by other species, particularly combative basidiomycetes. At later stages fungi with other characteristics may appear, e.g. those tolerant of nutrient stress, or able to obtain nutrition from the mycelia already present, or from by-products of their activity. Fruit body surveys hint that some fungi are associated with specific predecessors, i.e. they are more frequently found fruiting after certain species than after others. However, fruit bodies are a poor surrogate for active mycelium, and it has not been clearly determined whether the fungal species that arrive first dictate the subsequent pathway of community development, i.e. whether there is a priority effect at the species level. We used traditional culture-based techniques coupled with sequencing of amplified genetic markers to profile the communities in beech (*Fagus sylvatica*) disks that had been pre-colonised separately with nine species from various stages of fungal succession. Clear differences in community composition were evident following pre-colonisation by different species, with three distinct successor communities identified, indicating that individual species may have pivotal effects in driving assembly history. However, priority effects were strongly affected by forest site - the pattern was not the same on each one of the eight mixed deciduous forest experimental sites, which were geographically separated by between 0.25 and 105 miles. Priority effects may depend on the available air spora and soil-borne spores and mycelia, and may be linked to biochemical alteration of the resource and combative ability of the predecessor. Also, there was a strong correlation between fungal community structure and wood pH.

Common molds modify plant disease. Posy Busby[1], Kabir Peay[2], George Newcombe[3]. 1) Biology, Duke University, Durham, NC; 2) Biology, Stanford University, Stanford CA; 3) College of Natural Resources, University of Idaho, Moscow ID.

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Microfungi regarded as common molds (e.g., *Alternaria, Cladosporium*) occur in plant leaves as non-pathogenic leaf endophytes. Some endophytes are known to decrease or increase disease severity in their host plants, serving as defense mutualists or pathogen enablers, respectively. However, the generality of endophytes in modifying leaf disease severity has been explored rarely, and has not been coupled with studies of their abundance and distribution in wild populations. We used next-generation DNA sequencing to characterize the fungal leaf microbiome of *Populus trichocarpa* in wild populations throughout the Pacific Northwest (USA) and to determine how the relative abundance of common leaf endophytes correlated with the severity of a major leaf rust disease of *P. trichocarpa*, *Melampsora*. We

observed both positive and negative correlations between the relative abundance of common endophytes and rust severity. Using controlled inoculation experiment, we then confirmed that the endophytes modify rust disease severity in the predicted directions. Our results demonstrate that disease modification is a central function of common endophytes within the leaf microbiome of *P. trichocarpa* and that leaf endophytes help explain geographic variation in plant disease severity.

A highly diverse clade of melanized fungi associated with leaves and trichomes of the endemic tree *Metrosideros polymorpha* at high elevation sites in Hawai'i. Naupaka Zimmerman[1], A. Elizabeth Arnold[1,2], Peter Vitousek[3]. 1) School of Plant Sciences, University of Arizona, Tucson, AZ; 2) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 3) Department of Biology, Stanford University, Stanford, CA.

The Hawaiian Islands are among the most isolated landmasses on earth, separated by thousands of miles of ocean from the nearest continent. The youngest island in the Hawaiian chain, the Island of Hawai'i, is home to multiple volcanoes reaching 4000 m in elevation. This combination of isolation and high elevation creates a distinctive environment in which few plant species establish and grow. Of the hundreds of endemic tree species in the archipelago, only one grows at these highest elevation sites: *Metrosideros polymorpha* (Myrtaceae). As part of a culture-free study focused on the biogeography of foliar fungal endophyte communities, ITS1 pyrosequencing revealed unexpectedly high genetic diversity in a novel clade that was found almost exclusively at high elevation sites (1800m and 2400m elevation). Across a broad elevation gradient (100m to 2400m), endophyte species richness was generally higher at lower elevations, except for the portion of diversity represented by the novel clade: diversity in that lineage yields exceptionally high diversity at the highest sites. Well-supported phylogenetic reconstructions using Bayesian and Maximum Likelihood approaches place the clade in the Teratosphaeriaceae (Dothideomycetes). Subsequent culture-based investigations revealed that members of this clade live in close association with leaf trichomes of their hosts and grow exceptionally slowly in culture. Analysis at the population level provides insight into origins and distributions across the highest points on volcanoes of the Hawaiian Islands, and hypotheses regarding ecological modes in these unusual environments.

Invasiveness of the harmful house-invader *Serpula lacrymans* – population genomics of the Japanese and European populations. I. Skrede[1], J. Hess[1], S. V. Balasundaram[1], D. Eastwood[2], F. Martin[3], A. Kohler[3], C. Murat[3], D. Barry[4], M. Brandström Durling[5], H. Kauserud[1], N. Högberg[5]. 1) Department of Biosciences, University of Oslo, Oslo, Norway; 2) Department of Biosciences, Swansea University, Swansea, UK; 3) Interactions Arbres-Microorganismes, INRA-Lorraine University, Nancy, France; 4) Société Alcina, Montpellier, France; 5) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The dry rot fungus, *Serpula lacrymans*, is the most efficient decomposer of buildings in temperate regions worldwide. Population genetic data indicate that the species invaded Europe and Japan independently from its native range in central Asia. The European population has further dispersed to the Americas and Australia, and both the Japanese and European populations have reached New Zealand and there apparently admixed. In this study we are investigating the genetic, expressional and physiological basis for the success of the fungus as an invader of human-made wood constructions. Whole genome sequences are obtained from twenty strains from each of the two initial founder populations (Europe and Japan). Our preliminary results (based on two genomes from each population) show that the genetic diversity is higher in the Japanese population, as there is a tenfold difference in number of SNP detected within populations. This is also supported by previous studies using microsatellites and mating type linked markers. We also find that the Japanese strains are better competitors than the European strains (in our experimental set-up they displace the competitor in 60% vs. 45% of the encounters, respectively). In addition, preliminary results indicate that the Japanese population is more efficient in decomposing wood than the European population. We suggest that the better performance among the Japanese isolates are linked to the higher genetic variation found in this population.

Wednesday, March 18 3:00 PM–6:00 PM
Nautilus

Population Genomics and Microevolution
Co-chairs: Rachel Brem and Pierre Gladieux

The recent emergence of wheat blast in Brazil. Paulo C. Ceresini[2], Vanina L. Castroagudín[2], João L. N. Maciel[3], Bruce A. McDonald[1]. 1) Institute of Integrative Biology, ETH Zurich, Switzerland; 2) UNESP University of São Paulo State, Ilha Solteira, SP, Brazil; 3) EMBRAPA Wheat, Passo Fundo, RS, Brazil.

Wheat blast is caused by a *Pyricularia* species closely related to the rice blast fungus *Pyricularia oryzae*. It was first detected in the 1980s in Parana State, Brazil and has since spread across wheat growing areas in Brazil, Bolivia, Argentina and Paraguay. We conducted a series of studies to determine the population genetic structure of the wheat blast pathogen and elucidate its origins. We collected hierarchical samples of the fungus from wheat and other grass species across the known geographical range in Brazil, generating a sample of 553 isolates. These isolates were characterized for mating types, 11 SSR loci, sequences of 10 housekeeping genes, two genes associated with fungicide resistance, and an avirulence gene (*AvrCO39*). A subset of strains was phenotyped for virulence on a set of wheat differentials and resistance to QoI and azole fungicides. Since its emergence the wheat blast pathogen has evolved rapidly to become resistant to fungicides and specialized to include at least 14 pathotypes. The population genetic structure is consistent with a mixed reproductive system that includes regular recombination and significant gene and genotype flow over spatial scales of 1000s of km. By comparing sequences of 10 housekeeping genes in over 100 isolates, we found that the wheat blast and rice blast pathogens are closely

related, but phylogenetically distinct. We propose that the wheat blast pathogen should be named *Pyricularia tritici* to clearly distinguish it from the rice blast pathogen *Pyricularia oryzae*. Isolates of *Pyricularia* from poaceous hosts such as *Urochloa* grass were indistinguishable from *P. tritici* isolated from wheat and populations from the two host groups had the same genetic structure. Because *Urochloa* is a widely grown pasture grass occupying more than 90 million ha in Brazil, we propose that *Urochloa* provides a major source of wheat blast inoculum and may be the preferred host for pathogen recombination. We further postulate that *Urochloa* was the original host of *P. tritici* and that wheat blast emerged through a series of host jumps from *Urochloa* in Brazil.

Identification of candidate effectors in the poplar rust fungus *Melampsora larici-populina* through a population genomics approach. Antoine Persoons[1,2], Fabien Halkett[1,2], Stephane De Mita[1,2], Sebastien Duplessis[1,2]. 1) INRA, Unité Mixte de Recherche 1136 INRA/Université de Lorraine, Interactions Arbres-Microorganismes, 54280 Champenoux, France; 2) Université de Lorraine, Unité Mixte de Recherche 1136 INRA/Université de Lorraine IAM, 54506 Vandoeuvre-lès-Nancy Cedex, France.

The outcome of host-pathogen interactions depends on a complex molecular dialogue between the protagonists. Effectors released by the pathogen are critical for the success of infection, as they interfere with host metabolism, signaling and defense responses and allow expression of the disease. Effector proteins reported so far in rust fungi exhibit common features (e.g. secreted, small, cysteine-rich) and candidate effectors most likely reside among fungal secreted proteins. *Melampsora larici-populina* is a fungal pathogen responsible for the foliar rust disease on poplar trees, causing severe damage in plantations. Almost all the resistances (R) released so far have been overcome, with the latest major breakdown event in 1994 (R7). The genome of the virulent 7 isolate 98AG31 has been sequenced using a whole genome shotgun strategy, revealing a large genome of 101 megabases containing 16,399 predicted genes including 1184 small secreted proteins. A population genetics study based on 600 isolates was performed to finely determine the impact of this breakdown on the demographic history of *M. larici-populina.* The genomes of 80 poplar rust isolates, distributed among three genetic groups, were sequenced using Illumina technology to understand the effect of the R7 breakdown at the genetic scale. More than 300,000 polymorphic sites (SNPs) were uncovered across isolates, indicating a remarkable level of polymorphism. In order to understand the emergence of the virulence 7, we performed a genome scan analysis based on SNP data using differentiation and selection indices, taking into account the demographic history. We found several genomic regions related to the virulence 7 that bear genes encoding small secreted proteins. This study demonstrates the benefit of population genomics in the search for candidate effector genes. Functional validation of the most promising candidates is underway. AP is supported by the Lab of excellence ARBRE (ANR-11-LABX-0002-01) and the SFP.

Evolution of an outbreak: Hypermutators and the *Cryptococcus gattii* outbreak. R. Blake Billmyre[1], Shelly Clancey[1], Sheng Sun[1], Piotr Mieczkowski[2], Joseph Heitman[1]. 1) Duke University, Durham, NC; 2) University of North Carolina, Chapel Hill, NC.

Over the past fifteen years, an ongoing outbreak of the human fungal pathogen *Cryptococcus gattii* has occurred in the Pacific Northwest of the United States and Canada. This outbreak is comprised of three subtypes of the VGII molecular type of *C. gattii*, based on multilocus sequence typing, including the VGIIa/major, the VGIIb/minor, and the VGIIc/novel lineages. We performed whole genome sequencing and analysis of previously published genomes to analyze a total of 53 VGII isolates. Each of the clonal lineages underwent sexual recombination in the past, but recent crosses did not appear to contribute to the establishment of the outbreak lineages. Instead we found that VGIIa and VGIIb were likely introduced independently from South America and Australia respectively, while VGIIc may have arisen locally. Interestingly, we found that the VGIIa/major component of the outbreak has a clonal sublineage with diminished virulence that harbors a single base deletion in the coding region of the gene encoding the DNA mismatch repair component Msh2. Strains with this nonsense mutation in *MSH2* have an increased mutation rate, ~5-fold in typical genes, but an even more dramatic hypermutator phenotype (~100-fold elevation) in genes containing a homopolymer run within their coding regions. These genes occur frequently in the *C. gattii* genome, with 4% of the gene set containing a homopolymer run of 8 bases or longer in the coding region. One of these mutations in a homopolymer run resulted in unselected drug resistance to both FK506 and rapamycin via inactivation of the gene encoding the FKBP12 homolog. In a *de novo* deletion of *MSH2* and in crosses the hypermutation trait segregates with the *msh2* mutation. We hypothesize that the VGIIa/major strains responsible for the majority of the Pacific Northwest outbreak may have undergone microevolution mediated by a transient hypermutator state in adapting to a new environment to cause disease. This model has been previously demonstrated in virulences trajectories of bacterial pathogens and also operates in human colon cancer tumors but has not been previously observed in a fungal pathogen. Studies in progress seek to address whether the mutator state is an ancestral or derived lineage.

Adaptive genome remodeling by massive changes in gene content and gene transfers across cheese fungi. Antoine Branca[1], Jeanne Ropars[1], Ricardo Rodríguez de la Vega[1], Manuela López-Villaviciencio[2], Jérôme Gouzy[3], Erika Sallet[3], Sandrine Lacoste[2], Robert Debuchy[4], Joëlle Dupont[2], Émilie Dumas[1], Tatiana Giraud[1]. 1) Ecology Systematics and Evolution, CNRS - University Paris Sud, Orsay, France; 2) Institut de Systématique, Evolution, Biodiversité, UMR 7205 CNRS-MNHN-UPMC-EPHE, CP39, 57 rue Cuvier, 75231 Paris Cedex 05, France; 3) INRA-CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, Castanet-Tolosan, F-31326, France; 4) CNRS - Univ Paris-Sud, Institut de Génétique et Microbiologie UMR8621, Orsay, France.

Cheesemaking has been an essential innovation for the transformation of milk, a highly perishable food, into a long-term preservable product. Two key *Penicillium* species have been used for the maturing of cheese: *Penicillium roqueforti* for blue cheeses and *P. camemberti* for Brie-type cheeses. These two distantly related molds have been independently selected for growth in a human-made nutrient-rich environment. Little attention has focused on the domestication and strain improvement of fungi, with a few notable exceptions, despite their importance to bioindustry and to a general understanding of adaptation in eukaryotes. Here we compared the genomes of ten *Penicillium* species isolated from various environments, including five newly-sequenced genomes. We show that *Penicillium* fungi have adapted to the cheese medium through the convergent expansions of multigene families and multiple recent horizontal transfers of crucial metabolic genes. We found in cheese-making *Penicillium* high numbers of lineage-specific genes family expansions involved in the utilization of the nutrients present in the cheese, such as lactate and phospholipids. Most of these species-

specific gene expansions co-occurred in recently horizontally transferred regions, with almost 100% identity between distant species, and flanked by specific transposable elements. Laboratory assays linked the presence of a cheese fungus-specific horizontally-transferred region to both faster growth and greater competitiveness on cheese. Our results have both industrial and food safety implications, and improve our understanding of the genomic processes of adaptation to rapid environmental changes.

Exactly the same, except in every detail. Malcolm Whiteway, Hannah Regan, Yuan Sun, Pierre Cote. Biology, Concordia University, Montreal, Quebec, Canada.

Saccharomyces cerevisiae and *Candida albicans* represent two ascomycetes that have similar genomes and can be manipulated by transformation and molecular techniques, but have dramatically different lifestyles. In both the regulation of central metabolic circuits and in the control of specialized circuits such as those for mating, these two species use evolutionarily related proteins to perform functionally equivalent processes. However, analysis of these circuits identifies surprising variability in the way the cells actually use these similar proteins to do apparently similar jobs. We have been investigating the mating response pathway in the two species to understand how heterotrimeric G proteins and MAP kinase cascades are directed to control the response to mating pheromones. While the extensive rewiring of functions is currently being characterized in a variety of cellular processes, future research will need to be directed at understanding the evolutionary forces that have generated this variety.

Outcrossing limits propagation of chromosomal inversions in *Neurospora* species. Christopher Hann-Soden, John W. Taylor. Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

At least nine separate transitions from heterothallism to homothallism have occurred within the genus *Neurospora*. In each case the decreased gene flow associated with homothallism may have led to divergence of a new homothallic lineage. Theory predicts that highly inbred lineages should suffer from reduced efficacy of selection due to reduced genetic variation. The consequent degeneration of the genome would then lead to eventual extinction. Yet the abundance of homothallic lineages in groups such as *Neurospora* challenges this theory. We sought to determine the fate of homothallic lineages by comparing the published genomes of homothallic and heterothallic species along with a newly sequenced heterothallic species basal within the genus. Our whole genome approach allowed us to compare the incidence of chromosomal rearrangements as well as sequence variation. We found that heterothallic species from divergent lineages have fewer inversions relative to each other than are found between heterothallic and homothallic species, or between two homothallic species. Because inversions often reduce the fitness of heterozygotes (i.e. display underdominance), and haploid selfing as observed in homothallic species results in entirely homozygous tetrads, inversions that would be purged in heterothallics may persist in homothallics. Additionally, any deleterious inversions may persist in homothallics due to the reduced efficacy of selection on these populations. Together, the lack of selection on underdominant haplotypes and the reduced selection on deleterious haplotypes may lead to the propagation of inversions in homothallic species. Inversion events have been implicated in speciation processes by creating islands of reduced recombination, so this finding could have implications for the speciation of homothallic lineages. The propagation of inversions in a nascent homothallic population may further limit gene flow, accelerating the process of speciation and leading to radiation within homothallic lineages.

Coupling evolutionary dynamics of *Venturia inaequalis* effectors and functional genomics to decipher mechanisms of virulence and to identify durable resistance genes in apple. Benoit Calmes, Thibault Leroy, Adrien Biessy, Thomas Guillemette, Mélanie Sannier, Pascale Expert, Marie de Gracia, Aurélie Charrier, Jérôme Collemare, Valérie Caffier, Emilie Vergne, Elisabeth Chevreau, Charles-Eric Durel, Christophe Lemaire, Bruno Le Cam. IRHS-INRA, Beaucouzé, France.

During infection, pathogens secrete small secreted proteins (SSPs), called effectors, that promote disease. Plant receptors encoded by resistance *R* genes might recognize such effectors (also called avirulence factors *AVR*s), resulting in plant immunity. Pathogens evade recognition thanks to the emergence of virulent alleles present in populations. It has been demonstrated that avirulent effectors are crucial for the pathogen infection cycle and that their loss-of-function may induce a substantial fitness cost. This kind of effector is expected to be under purifying selective pressure. Here, we aim at identifying the effector repertoire of *Venturia inaequalis*, the agent of apple scab, assessing its evolutionary dynamics and studying the role of candidate effectors in virulence. We sequenced *de novo* 90 strains, collected on apple and on their wild relatives and differing in their host range or virulence to study allelic polymorphism at 880 putative effector loci. The top-20 hits for highly conserved sequences were selected as candidates for further functional analyses. *In planta* gene expression showed a significant induction of these conserved SSP at the early stage of plant infection. Their functions were investigated using targeted deletion mutants. Remarkably, loss of two conserved SSPs resulted in reduced aggressiveness without any alteration in growth *in vitro*. GFP-tagged protein and heterologous expression were used to assess their sub-cellular localization in infected apple leaves. Involvement of theses SSP in the modulation of host defence was also investigated using an apple full-transcript microarray. Highly conserved effectors will be used to screen for novel *R* genes in *Malus* genotypes characterized for their high resistance to scab. This combined knowledge should enable us to understand strategies used by the pathogen to overcome defences in apple and consequently to build more durable resistance towards apple scab.

***Saccharomyces* diversity and the tools to tap it.** Chris T. Hittinger. Laboratory of Genetics, Genome Center of Wisconsin, DOE Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, WI.

Saccharomyces cerevisiae is one of the most thoroughly studied model organisms, but other members of the genus have been characterized minimally. Until recently, most *Saccharomyces* species have been known only through their genetic contributions to industrial interspecies hybrids or by a handful of wild isolates. Improved isolation techniques have dramatically expanded strain collections to enable population genetic analyses. Here, using whole genome sequencing, we examine the diversity of the early-branching sister species, *Saccharomyces eubayanus* and *Saccharomyces uvarum*. Population and phylogenomic analyses support the existence of multiple

well-differentiated populations of each species, as well as rare mosaic lineages. These species are represented by a total of four diverse sympatric populations in Patagonia, South America, while rare Northern Hemisphere isolates related to these populations have comparatively low sequence diversity, are natural intraspecies hybrids or mosaics, or are interspecies hybrids used in the production of beer, wine, or cider. Several possible biogeographical and domestication scenarios are discussed. As more natural *Saccharomyces* strains become available for study, general genome manipulation strategies are needed for wild prototrophic strains. We have recently developed a novel cassette for high-throughput genome engineering that deploys an inducible double-strand break generator and selectable/counterselectable marker. This technique enables pooled allele replacement and mutagenesis with efficiencies higher than those reported for CRISPR/Cas9-based systems. We show its utility across the genus *Saccharomyces* and provide a roadmap for experimentally testing the functional meaning of sequence diversity.

Wednesday, March 18 3:00 PM–6:00 PM
Scripps

Synthetic Biology
Co-chairs: Ken Bruno and Debbie Yaver

How to modify regulatory proteins for desired gene expression? Christian Derntl[1], Thiago Mello-de-Sousa[1], Daniel Kiesenhofer[1], Alice Rassinger[1], Marcio Poças-Fonseca[2], Robert Mach[1], Astrid Mach-Aigner[1]. 1) Vienna University of Technology, Vienna, Austria; 2) University of Brasilia, Brasília, Brazil.

During evolution *Trichoderma reesei* adopted a saprophytic lifestyle that is sustained by enzymatic degradation of plant cell walls. The fungus is able to secrete these enzymes in high amounts and is therefore used for industry-scale production of in particular cellulases. Consequently, research efforts focused on the one hand on the increasingly efficient expression of cellulases, and on the other hand, on the possibility of exploiting the high-secreting potential for production of other proteins. Studies on the transcriptional regulation of the genes encoding the plant cell wall degrading enzymes led to the identification of a number of regulatory proteins: for example Xyr1, which is the main and essential transactivator. Otherwise, Cre1 confers carbon catabolite repression of target gene expression, while Xrp1 acts more like a narrow domain transcription factor and represses expression of only a part of the mentioned genes. Amongst other regulatory proteins, these transcription factors act either directly on the expression of target genes or influence their chromatin packaging. Certain modifications of these regulatory proteins, starting from a single amino acid exchange to the loss of complete domains, provoke changes in their function, and finally, in the expression of their target genes. Examples will be provided that best possible knowledge about the (domain) composition of these regulatory proteins opens the road for efficient control of their target gene expression.

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Engineering *Neurospora crassa* for increased production of lipids from lignocellulose. Christine Roche[1,3], Douglas Clark[1,3], Louise Glass[2,3]. 1) Chemical and Biomolecular Engineering, UC Berkeley, Berkeley, CA; 2) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 3) Energy Biosciences Institute, UC Berkeley, Berkeley, CA.

Microbially-produced triacylglycerol (TAG) is a potential source of oil for the production of biodiesel, but commercialization will require high TAG yields from low-cost renewable feedstocks. Lignocellulosic biomass has been highlighted as a viable feedstock for microbial biofuel production due to its abundance and low cost; however, liberation of fermentable sugars from lignocellulose is a major limiting factor for an economically viable biofuel. We propose to overcome this limitation using the cellulolytic filamentous fungus, *Neurospora crassa*, as a whole-cell biocatalyst to directly convert lignocellulose to TAG. The present study employs a multi-gene approach for increasing lignocellulose-derived TAG biosynthesis in *N. crassa*. We demonstrate a 2-fold increase in TAG production and accumulation in *N. crassa* by redirecting carbon flux from glycogen biosynthesis towards fatty acid biosynthesis (glycogen synthase knockout, Δ*gsy-1*) and by relieving fatty acyl-CoA ester feedback inhibition of fatty acid biosynthesis (acyl-CoA synthetase knockout, Δ*acs-3*). Furthermore, stacking these mutations in an enhanced cellulose degrading strain of *N. crassa* (Δ*acs-3* Δ*cre-1;* Δ*gsy-1*) enabled up to 4-fold increase in lignocellulose-derived lipid production above the wild-type strain.

Reconstruction of the biosynthetic pathway for the terpene antibiotic pleuromutilin in the secondary host *Aspergillus oryzae*. Fabrizio Alberti[1], Colin M. Lazarus[1], Chris L. Willis[2], Andy M. Bailey[1], Gary D. Foster[1]. 1) School of Biological Sciences, University of Bristol, Bristol, United Kingdom; 2) School of Chemistry, University of Bristol, Bristol, United Kingdom.

Pleuromutilin is an antibiotic that is produced as a secondary metabolite by the basidiomycete fungus *Clitopilus passeckerianus*. Pleuromutilin is a tricyclic diterpene and has been exploited as a precursor for many semi-synthetic antibiotics, one of which – Retapamulin – is currently used for the treatment of Impetigo and other serious skin infections. However *C. passeckerianus* produces pleuromutilin in low amounts, strain improvement and manipulation of the fungus are made problematic by its dikaryotic nature, and total synthesis of the antibiotic has only been achieved with low yields.

In order to increase the production titre of pleuromutilin and to fully exploit this new growing class of natural product antibiotics, the biosynthetic pathway for pleuromutilin was reconstructed in the heterologous host *A. oryzae*, which has a GRAS status and is amenable to growth in industrial fermenters. Multigene expression vectors were used to obtain transformation of *A. oryzae* with the genes of the pleuromutilin cluster. Identification of the metabolites produced was achieved through analytical chemistry techniques, such as HPLC, NMR Spectroscopy and High Resolution Mass Spectrometry.

A synthetic biology approach was used to achieve heterologous biosynthesis of pleuromutilin in *A. oryzae*. An enhanced titre of the antibiotic was ultimately established in the heterologous host, tenfold over the natural host producer *C. passeckerianus*. The function of each enzyme in the pathway is being uncovered through expression of different combinations of genes in *A. oryzae* and consequent isolation of metabolites. This strategy also allowed to isolate a previously undescribed intermediate involved in biosynthesis of the antibiotic pleuromutilin.

Metabolic pathway engineering for organic acid production in *Aspergillus niger*. Peter Punt[1], Abeer Hossain[1,2]. 1) Microbiology & systems Biology, TNO, Zeist, Netherlands; 2) University of Amsterdam, the Netherlands.

Among the compounds listed as top building blocks chemicals in particular organic acids have gained industrial interest for biobased production. Many of these, also being food ingredients, are directly derived from the central metabolic pathway in every living cell, the tricarboxylic acid (TCA) cycle. The volume-wise largest biotechnologically produced compound, besides ethanol, is citric acid, which is a precursor for most of the other organic acids. As the yields for citric acid in *Aspergillus niger* are close to the theoretical yield, the citric acid pathway is a promising backbone for organic acid production using genetic engineering. Based on these considerations we embarked on a research program for producing itaconic acid using A. niger. For *A. niger* this acid, which is produced in very few human- or plant-pathogenic fungi, is a non-native product.

At the start of our research, we elucidated the genetic basis of the biochemical pathway for itaconic acid. Using a transcriptomics approach, we did not only identify the biochemical pathway gene but also the genes encosing the mitochondrial and plasma membrane transporters, confirming that the pathway in the native host *A. terreus* is compartmentalized. Overexpression showed that all three were relevant for itaconic acid overproduction in *A. niger*. Molecular genetic and process technological research performed on the *A. niger* itaconic acid-producing strains resulted in further improvement of the itaconic acid titres and the reduction of by-product formation. Array analysis in *A. terreus* and RNAseq transcription analysis in the recombinant *A. niger* strains, revealed the potential relevance of the pentose phosphate pathway and a hitherto unidentified gene cluster related to organic acid production. Overexpression of a gene from this latter gene cluster allowed further increase in itaconic acid titers and reduced citric acid coproduction.

References: Li, A. et al. (2011) Fungal Genetics and Biology 48, 602-611, Li, A. et al. (2013) Applied Microbiology and Biotechnology 97, 3901-3911.

A CRISPR/Cas9 system for genetic engineering of filamentous fungi. Christina S Noedvig, Jakob B. Nielsen, Uffe H. Mortensen. DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark.

The harnessing of the prokaryotic and archaeal immune mechanism CRISPR (clustered regularly interspaced short palindromic repeats) as a tool for genetic engineering in eukaryotes, has proved to be a powerful technology. CRISPR/Cas9 introduces specific DNA double strand breaks (DSB) with high precision, which in turn can be employed to efficiently stimulate gene targeting. Consisting of two components, an RNA guided nuclease Cas9 and a chimeric guide RNA (gRNA), a specific DSB can be produced in the host organism. The cleavage target site is determined by 20 base pairs (bp) in the gRNA, and by exchanging those 20 bp, Cas9 can be programmed to target a specific chromosomal location with few constraints. The technology has had a huge impact on genetic engineering of organisms, such as plants or mammalian cells where gene targeting is notoriously inefficient, but has so far not been adapted to filamentous fungi. Low gene targeting frequencies is a common problem when attempting to do gene editing in filamentous fungi. A common strategy to circumvent this problem is to delete or disable one of the key genes in the non-homologous end-joining (NHEJ) pathway to greatly enhance gene-targeting frequencies. However, for fungi where a genetic toolbox is not in place, the initial establishment of genetic markers and NHEJ-deficiency can be laborious. Here we present a CRISPR/Cas9 system adapted for filamentous fungi and show that it can be efficiently used to introduce specific genomic modifications. Considering that the number of fully sequenced fungi is dramatically increasing, and that the vast majority of these fungi does not possess a genetic toolbox, our system will be a highly useful in developing the initial marker- and NHEJ gene mutations to establish such a toolbox. To this end, we have also developed a gRNA design software that facilitates identification of gRNA sequences that can target a desired gene in several different species, hence, reducing the plasmid construction workload. Together, we envision that our tools can be used to rapidly expand the repertoire of fungi where genetic engineering is possible and therefore greatly accelerate the exploration of fungal biology.

High-efficiency genome editing and allele replacement in prototrophic and wild strains of *Saccharomyces*. William Alexander[1,2], Drew Doering[1,3], Chris Hittinger[1,2,3]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI; 3) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

Current genome editing techniques available for *Saccharomyces* yeast species rely on auxotrophic markers, limiting their use in wild and industrial strains and species. Taking advantage of the ancient loss of thymidine kinase in the fungal kingdom, we have developed the

herpes simplex virus thymidine kinase gene as a selectable and counterselectable marker that forms the core of novel genome engineering tools called the Haploid Engineering and Replacement Protocol (HERP) cassettes. Here we show that these cassettes allow a researcher to rapidly generate heterogeneous populations of cells with thousands of independent chromosomal allele replacements using mixed PCR products. We further show that the high efficiency of this approach enables the simultaneous replacement of both alleles in diploid cells. Using these new techniques, many of the most powerful yeast genetic manipulation strategies are now available in wild, industrial, and other prototrophic strains from across the diverse *Saccharomyces* genus.

***Aspergillus nidulans* as cell factory for production of mycophenolic acid.** Zofia D. Jarczynska[1], Jakob B. Nielsen[1], Freja Aasted[1], Dorte M. K. Holm[2], Kiran R. Patil[3], Kristian F. Nielsen[1], Uffe H. Mortensen[1]. 1) Systems Biology, DTU, Kgs. Lyngby, Denmark; 2) Novozymes A/S, Denmark; 3) European Molecular Biology Laboratory, Heidelberg, Germany.

Filamentous fungi are well-known producers of a wide range of valuable secondary metabolites (SMs), which can be advantageously exploited e.g. in pharmaceutical industry. One of the most prominent examples is mycophenolic acid (MPA), an immunosuppressant molecule that inhibits inosine-5'-monophosphate dehydrogenase (IMPDH). IMPDH catalyzes the rate limiting step in the guanine nucleotide synthesis in B- and T-lymphocytes. Recent studies have successfully identified the gene cluster, coding for the MPA synthesis, in *Penicillium brevicompactum*. Moreover, it has been demonstrated that two first steps in MPA production are catalysed, respectively, by polyketide synthase (PKS), MpaC, producing 5-methylorsellinic acid (5-MOA), and MpaDE, which strikingly is a natural fusion enzyme catalysing the production of 5,7-dihydroxy-4-methylphtalide (DHMP). Additionally, *mpaF* has been characterized as IMPDH-encoding gene which confers the resistance to MPA. In order to characterize the remaining part of the MPA biosynthetic pathway, we have heterologously expressed the *mpa* cluster genes in a stepwise manner in *Aspergillus nidulans*. We have demonstrated that MpaA possesses prenyl transferase activity and catalyzes the conversion from DHMP to 6-farnesyl-5,7-dihydroxy-4-methylphtalide (FDHMP). To our surprise, this strain was also able to produce demethyl-MPA, which is the next intermediate in MPA biosynthesis. Interestingly, we have found two homologs of *mpaH* in *A. nidulans*, which is hypothesized to encode the conversion of FDHMP to demethyl-MPA, and we speculate that one or both of these genes deliver hydrolase activity similar to the one encoded by MpaH. Lastly, we have confirmed that MpaH and MpaG catalyze the last two enzymatic steps in the biosynthesis of MPA, resulting in the production of demethyl-MPA and MPA, respectively. In conclusion, we have successfully characterized the full biosynthetic pathway of the top-selling drug, MPA. Moreover, we have demonstrated that *A. nidulans* is a suitable cell factory for heterologous production of MPA.

Computational modelling of *Aspergillus* metabolism for cellular engineering purposes. Blaine Pfeifer. University at Buffalo, Buffalo, NY.

In this work, we describe computational metabolic engineering models to identify genetic manipulation targets for improved highly-reduced polyketide formation from *Aspergillus* spp. Specifically, Flux Balance Analysis (FBA) has been utilized together with a metabolic network model in coordination with an algorithm termed Minimization of Metabolic Adjustment (MoMA) to identify gene deletions predicted to improve specific metabolite overproduction. In addition, a separate modeling approach termed Elementary Mode Analysis (EMA) was used as a complement to the genome-scale FBA approach. In EMA, a smaller metabolic network, composed mostly of primary metabolism, is used to predict beneficial gene deletions without the need to utilize an objective function (as is required for FBA). As such, EMA provides an alternative approach to predictive modeling, and both approaches were undertaken to maximize the potential for identifying targets that improve final production metrics experimentally. Top targets for both modeling strategies will be described as well as current modeling approaches designed to complement experimental efforts by collaborators.

Thursday, March 19 3:00 PM–6:00 PM
Merrill Hall

Fungus-plant interactions
Co-chairs: Natalia Requena and Wilhelm Schäfer

Biotrophic transportome in the arbuscular mycorrhiza. Daniel Wipf[1], Leonardo Casieri[1], Carole Pfister[1], Nathalie Leborgne-Castel[1], Nassima Ait Lahmidi[1], Joan Doidy[1], Laurent Bonneau[1], Pierre Emmanuel Courty[2]. 1) UMR Agroecologie, INRA/AGrosup/Burgundy University, Dijon, France; 2) Botanical Institute, University of Basel Hebelstrasse 1 CH-4056 Basel, Switzerland.

Understanding mechanisms underlying high nutrients use efficiency and carbon allocation in a context of mycorrhizal interactions is critical for sound management of croplands taking care of ecosystem services rendered by mycorrhizal fungi. Transport processes across the polarised membrane interfaces are of major importance in the functioning of the established mycorrhizal association as the symbiotic relation is based on a 'fair-trade' between fungus and host plant. Uptake and exchanges of nutrient and/or metabolites, at biotrophic interfaces are controlled by membrane transporters and their regulation patterns are essential in determining the outcome of plant fungal interactions and in adapting to changes in soil nutrient quantity and/or quality. The talk will present the current state of art with a special focus on S and C transports.

Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. Jessica Soyer[1], Mennat El Ghalid[1], Jonathan Grandaubert[1], Marie-Hélène Balesdent[1], Lanelle Connolly[2], Michael Freitag[2], Thierry Rouxel[1], Isabelle Fudal[1]. 1) INRA, UR1290 BIOGER, Grignon, France; 2) Biochemistry and Biophysics Dept, Oregon State University, Corvallis, USA.

Plant pathogens 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 during plant infection. To date, little is known about the regulation of their expression. *Leptosphaeria maculans* is an ascomycete fungus responsible for stem canker of oilseed rape. Its genome has a bipartite structure alternating gene rich GC-equilibrated 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 one histone modification, histone H3 lysine 9 methylation (H3K9me3), in epigenetic regulation of concerted effector gene expression in *L. maculans*. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, HP1 and DIM5, by RNAi. By using HP1-GFP as a heterochromatin marker, we observed that almost no chromatin condensation is visible in a silenced-*dim5* background. Whole genome oligoarrays performed on silenced-*hp1* and silenced-*dim5* transformants background revealed an over-expression of pathogenicity-related genes during *in vitro* growth, with a favored influence on SSP-encoding genes in AT-isochores. That increase of expression during *in vitro* growth was associated with a reduction of H3K9 trimethylation at two SSP-encoding gene loci. These data strongly suggest that an epigenetic control, mediated by HP1 and DIM5, represses the expression of at least part of the effector genes located in AT-isochores during growth in axenic culture. Our hypothesis is that changes of lifestyle and a switch toward pathogenesis lift chromatin-mediated repression, allowing a rapid response to new environmental conditions.

Get ready for infection: Transcriptional profiling reveals virulence-specific traits inside of infection cushions of *Fusarium graminearum*. Jörg Bormann[1], Marike J. Boenisch[1], Anika Glasenapp[1], Ana-Lilia Martinez Rocha[1], Stefan Scholten[2], Sebastian Piehler[3], Martin Münsterkötter[3], Ulrich Güldener[3], Bernard Henrissat[4], Marc-Henri Lebrun[5], Wilhelm Schäfer[1]. 1) Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Germany; 2) Biocenter Klein Flottbek, Developmental Biology, University of Hamburg, Germany; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Germany; 4) UMR 7257 - Centre National de la Recherche Scientifique & Aix-Marseille Université Case 932, France; 5) UMR 1290 - INRA AgroParisTech BIOGER-CPP, Versailles, France.

The fungal pathogen *Fusarium graminearum* forms specialized infection cushions (ICs) essential for penetration of wheat floral-leaf cells. To understand the molecular basis of IC development, ICs and non-invasive runner hyphae (RH) were isolated by laser capture microdissection and subjected to RNAseq. Quantitative expression analysis show marked differences in gene expression patterns between RH and ICs:1. The majority of known and putative secondary metabolite gene clusters, including those responsible for trichothecene and butenolide production, are significantly up-regulated in ICs, 2. Carbohydrate-modifying enzymes (CAZymes) with proven capacities for cell-wall degradation are exclusively present in ICs. In total, 174 genes encoding for CAZymes are differentially expressed (42 in RH, 132 in ICs), 3. Genes encoding for enzymes involved in reactive-oxygen species metabolism reside in the upper ranks of differentially expressed genes (DEGs). Secreted ROS-related enzymes (SREs), presumably involved in plant-defense response, are relatively enriched in ICs, 4. We identified a large subset of transcripts encoding for putative effector proteins. By use of this novel transcriptional profiling of runner hyphae and infection cushions from a fungal plant pathogen obtained under in planta conditions, we gain new insights in the initial infection process of *F. graminearum* on wheat. Complementary to this approach, functional characterization of genes and histological analyses are ongoing. First results will be presented. We conclude that infection cushions serve as an armory of virulence factors.

Necrotrophic effector epistasis in tan spot of wheat. L.M. Ciuffetti, V.M. Manning, I. Pandelova. Dept Botany & Plant Pathology, Oregon State Univ, Corvallis, OR.

Pyrenophora tritici-repentis, the causal agent of tan spot disease of wheat, has been identified in major wheat growing areas worldwide and tan spot is considered a disease of economic importance. Tan Spot has emerged as an experimental model for the study of diseases that conform to an inverse gene-for-gene relationship where pathogenicity/virulence has been causally associated with the production of multiple host-selective toxins (HSTs). The importance of HSTs in disease development indicates that the loss of any of these pathogenicity factors would cause a reduction in virulence when the pathogen expresses multiple HSTs to which the host is sensitive. However, following deletion opf the gene encoding the HST Ptr ToxA (ToxA) or the heterologous expression of *ToxA* in a race that previously did not produce this toxin, we demonstrate that ToxA symptom development is epistatic to other HST-induced symptoms. Data from this study will be presented and indicate a complex interaction between host responses and at least some HSTs. To our knowledge, this is the first demonstration of necrotrophic effector epistasis.

Functional characterization of CgEP2, a broadly conserved fungal effector with a role in virulence in *Colletotrichum graminicola*. José M. Sanz-Martín, Walter Vargas, Vinicio Armijos-Jaramillo, Michael R. Thon, Serenella A. Sukno. Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, 37185 Villamayor, Spain.

During infection process, fungal pathogens secrete a wide range of enzymes and effector proteins to interact with their hosts and manipulate the plant immune system. To resist pathogen invasion, plants induce a large battery of defenses including PR proteins production such as chitinases. Recently, several fungal effectors have been identified that interfere with chitin-triggered immunity, protecting the fungal hyphae against hydrolysis by chitinases or sequestering the chitin oligosaccharides and preventing chitin from binding to the receptor. In this study, we describe CgEP2 (*Colletotrichum graminicola* Effector Protein 2), a 640 aa secreted protein in the maize pathogen *C. graminicola*, with a role in virulence. CgEP2 is a Zn dependent metalloprotease of the fungalysin family. Members of this family have been shown to bind to plant produces class IV chitinases (PR-4) and induce post-translational modifications. Phylogenetic analysis shows that CgEP2 is, highly conserved in diverse pathogenic fungi. Quantitative PCR (qPCR) assays using different time points during leaf anthracnose as well as transcriptional fusions of the gene promoter with a GFP cassette show that gene expression is activated at the late biotrophic stage, specifically when the fungus switches to necrotrophic growth. To confirm it's role in pathogenesis, we constructed null mutants tagged with GFP by gene replacement using Delsgate methodology and performed pathogenicity assays in maize. The null

mutant develops reduced lesion sizes on leaves and reduced colonization of roots, showing that CgEP2 has a role in *C. graminicola* virulence. Also, biochemical analysis of chitinase activity in leaves infected with null mutant confirms the lack of ability to degrade this substrate. Our results show that CgEP2, which is a broadly conserved fungal effector, plays a role in plant infection and host colonization and could be important in the lifestyle change of *C. graminicola*.

Effectors of *Fusarium oxysporum*: identification, function, evolution and regulation of gene expression. Martijn Rep, Sarah Schmidt, Peter van Dam, Mara de Sain, Ido Vlaardingerbroek, Shermineh Shahi, Sri Widinugraheni, Like Fokkens, Nico Tintor, Bas Beerens, Petra Houterman, Charlotte van der Does. Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands.

Small proteins secreted by plant-pathogenic fungi during host colonization are often called 'effectors', especially when a role in plant colonization or disease symptom induction has been established. The *Fusarium oxysporum* species complex (FOSC) harbors a striking diversity of highly host-specific pathogenic strains, mainly causing vascular wilt diseases. Genome compartmentalization into a 'core' and 'accessory' genome is a general feature of the FOSC. The accessory genome harbors all effector genes and is very rich in transposons. We would like to know to what extent the remarkable host-specificity within the FOSC can be attributed to specific suites of effector genes, how these genes evolve and spread within the FOSC, and how their expression is regulated.

Through xylem sap proteomics and comparative genomics we have obtained a comprehensive overview of the suites of effector genes in a diverse set of strains that infect tomato, cucumber, melon or watermelon. The cucurbit-infecting strains, belonging to *forma specialis cucumerinum*, *radicis-cucumerinum*, *melonis* or *niveum* have overlapping but distinct effector suites. Almost all effector genes of tomato-infecting strains (*forma specialis lycopersici*) reside on a single accessory chromosome that can be transferred between strains through an as yet unknown mechanism. Loss of this chromosome leads to complete loss of pathogenicity, while at least half of the chromosome, including several effector genes, is dispensable for pathogenicity.

Expression of effector genes in tomato-infecting strains requires a conserved transcription factor encoded in the core genome (SGE1), and is also regulated by FTF1, a transcription factor encoded on the accessory genome [1]. FTF1 is upregulated during infection and binds to an element that is present in the promoters of several effector genes.

[1] de Vega-Bartol *et al.* (2011) Phytopathology 101: 470-479.

Towards deciphering the functional role of arbuscular mycorrhizal effectors in the symbiosis. Natalia Requena, Ruben Betz, Meike Hartmann, Sven Heidt, Leonie Hacker. Molecular Phytopathology, Karlsruhe Institute of Technology, Karlsruhe, Germany.

Arbuscular mycorrhizal (AM) fungi colonize plant roots and provide plants with mineral nutrients, prominently phosphate, in exchange for carbohydrates. The mutualistic symbiosis is one of the most widespread and ancient on the earth. The wide host range of AM fungi and the conserved key features of the association with all involved plants (arbuscule formation, modulation of phosphate homeostasis and reprogramming of carbon partitioning) suggests that identical molecular mechanisms underlie the functioning of the symbiosis. With our discovery of the first AM fungal effector protein SP7 (Kloppholz et al., 2011) the paradigm that AM fungi are naïve colonizers of plants and that the symbiosis is exclusively controlled by the plant was challenged. We showed that SP7 modulates the MAMP triggered immunity of the plant and thus promotes biotrophy. This suggests that in all other AM symbioses, involving other AM fungal species and other plants, similar or identical mechanisms might operate. In addition, we predict that other effectors controlling additional symbiotic features must exist. With the recent outcome of the first AM fungal genome (Tisserant et al., 2013; Lin et al., 2014), novel effector candidates have been identified and their functional characterization is in progress. We have focussed our efforts towards effectors containing nuclear localization domains, including two prominent effector families, the SP7-like family and the Crinkler family, and a couple of few other proteins. The existence of the Crinkler effector family in AM fungi is interesting *per se*, as this effector family is only found in Oomycetes and Chytridiomycetes. Despite their name and the phenotype some of them induce when expressed *in planta* (crinkled leaves), some Crinkler effectors have been shown to supress cell death. The SP7-like family and the Crinkler family have share in addition the ability to enter the plant cell on their own. And while a putative entry motif has been ascribed for Crinkler proteins, the mechanism by which SP7-like proteins enter the plant cell is yet unknown. Here we will present our latest results concerning the functional characterization of AM fungal effectors and their role in symbiosis.

RNAse-like effectors of cereal powdery mildews. Pietro Spanu, Helen Pennington, Rhian Jones, Michal Przydacz, Ernesto Cota, Tolga Bozkurt. Dept Life Sci, Imperial Collge London, London, BE., United Kingdom.

The genomes of the fungi that cause powdery mildews in cereal grasses encode over 500 secreted effector proteins. About a quarter of these are short proteins that bear some similarities to microbial RNAses. The expression of these genes is associated with the development of haustoria. In past work we demonstrated that in the barley powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, BEC1011 and BEC1054, are RNAse-Like Proteins in Haustoria (RALPH) are effectors necessary for full pathogenicity. Moreover, the expression of BEC1054 in plants other than the barley host leads to an increase in susceptibility to adapted pathogens. The mildew RALPH effectors are therefore one of the principal effector families in these fungi. We have determined that the 3D structure of BEC1054 is highly similar to that of RNAses but lacks enzyme activity. BEC1054 interacts with nucleic acids, but also with host proteins. Here I will propose possible models that explain the mode of action of these effectors, why this diversity has arisen and is maintained.

Thursday, March 19 3:00 PM–6:00 PM
Chapel

Gene regulatory networks
Co-chairs: Audrey Atkin and Gerhard Braus

Post-transcriptional regulation of gene expression by upstream open reading frames. G. Newcomb[1], E. Choi[2], K. Sayood[1], A. Atkin[2]. 1) Department of Electrical Engineering, University of Nebraska-Lincoln, Lincoln, NE; 2) School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.

Regulation of gene expression controls cell fitness, response to environmental changes and development. This regulation is the combined effect of transcriptional and post-transcriptional regulation. Post-transcriptional regulation has a profound effect on overall expression levels. For example, gene-specific differences in translation efficiency vary by over 100-fold and mRNA half-lives range from a few minutes to hours. These differences are determined by innate mRNA features as well as interactions between individual mRNAs and proteins or noncoding RNAs. Because post-transcriptional regulation has a substantial, if not equal, impact on gene expression levels, it is important to understand the mechanisms that regulate mRNA function and integrate this information into gene regulatory networks. Upstream open reading frames (uORFs) are an innate mRNA feature that affects translation and mRNA stability. uORFs are short open reading frames with a start codon located upstream of the main open reading frame (ORF). They reduce protein expression levels by acting as a decoy for the translation initiation machinery resulting in reduced translational initiation of the main downstream ORF. uORFs also act as a targeting signal for rapid mRNA decay. However not all uORFs affect translation or mRNA stability. Thus, we do not yet have a complete understanding of the features affecting uORF function. Recent technical advances in mRNA sequencing have improved genome-wide annotation enabling more accurate detection of uORFs, and ribosome profiling now makes it possible to assess uORF function. We have used this new information to compile a database of *Saccharomyces cerevisiae* uORFs and determined how distance to the cap, uORF start codon context and relative location of the uORF affect protein production and mRNA stability. We are using this information to build a mathematical model that can predict the effect of uORFs in native mRNAs on gene expression as well as enable design of synthetic mRNAs with uORFs tuned to produce precise amounts of protein.

Evolution of Fungal Pathogenesis. Alexander Johnson. Microbiology and Immunology Dept, UCSF, San Francisco, CA.

Many of the differences among species are due to changes in gene expression programs rather than differences in gene content. We have reconstructed the evolutionary history of several transcriptional circuits across the ascomycetes, which include *Saccharomyces* and *Candida* species, and nominally represent 300 million years of diversification. Although the DNA-binding specificity of transcription regulators are often preserved over these evolutionary times, the connections between regulators and the genes they control change rapidly. The ease of these wiring changes results from several basic features of transcription regulation, including regulatory protein modularity, cooperative binding, and the low information content of cis-regulatory sequences. Some wiring changes provide novel phenotypes, while others seem to preserve ancestral circuit output but alter the structure of the circuit through which that output is achieved. Along the *C. albicans* lineage, many rewiring changes have led to the formation of relatively recent circuits that enable *Candida* species to thrive in mammalian hosts. These include circuits that control biofilm formation, proliferation in the host, white-opaque switching and other properties associated with pathogenesis.

Metals, Metabolomes and Circuitry Networks. Nancy Keller[1], Philipp Wiemann[1], Fang Yun Lim[1], Joshua Baccile[2], Frank Schroeder[2]. 1) Dept Med MicroBiol & Dept Bact, Univ Wisconsin, Madison, Madison, WI; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, United States.

We have recently reported on an iron-responsive secondary metabolite pathway in *Aspergillus fumigatus*[1], where the iron binding non-ribosomal peptide hexadehydroastechrome (HAS) was central to a feedback circuitry balancing iron pools in the fungus in coordination with secondary metabolite synthesis. In binding iron, HAS causes an iron starvation phenotype. Here we find HAS also has the ability to bind copper, resulting in transcriptional profiles suggestive of copper starvation. We uncover copper responsive circuitries, including a copper-responsive secondary metabolite gene cluster, and evidence of copper-iron crosstalk. We provide a model of secondary metabolites as components in maintaining metal homeostasis in *Aspergillus*.

[1]Front Microbiol. 2014 Oct 24;5:530.

Impact of heterogeneity on gene regulatory networks. Han Wosten, Fengfeng Wang, Pauline Krijgsheld. microbiology, utrecht university, utrecht, Netherlands.

Fungi form colonies that consist of a network of hyphae. Macro-colonies with a diameter > 1 cm are formed on solid media, while micro-colonies with a diameter in the mm-scale are formed in liquid shaken cultures such as bioreactors. In general, RNA is isolated from whole cultures or macro-colonies to study gene regulatory networks. During the last two decades however it has become clear that fungal colonies within a liquid culture are heterogeneous in gene expression. In fact, heterogeneity in RNA composition can even be found between and within zones of macro- and micro-colonies. These findings imply that gene regulatory networks will not be correctly predicted by using whole cultures and colonies. This will be illustrated by expression of genes involved in asexual reproduction and in metabolism of xylose and starch. For instance, inactivation of the sporulation gene *flbA* of *A. niger* does not impact *brlA* mRNA levels when RNA is extracted from whole colonies. Yet, there is an effect on expression of this master regulator of asexual development when zones of wild-type and

Δ*flbA* cultures are compared. Transcripts of *brlA* are absent in all zones of wild type *A. niger* colonies and in the intermediate and outer zones of the Δ*flbA* colonies. In contrast, *brlA* is expressed in the center of Δ*flbA* colonies. This indicates that FlbA is part of the *brlA* regulatory network in the central zone of *A. niger* colonies but not in its middle and outer zones.

Histidine kinase pathway components are required for growth in the parasitic form of *Histoplasma capsulatum*. Sinem Beyhan[1], Giselle Knudsen[1], Anita Sil[1,2]. 1) Microbiology and Immunology, Univ California, San Francisco, San Francisco, CA; 2) Howard Hughes Medical Institute.

Histoplasma capsulatum is a respiratory fungal pathogen of humans and has a dimorphic life cycle, switching from an infectious filamentous form in the soil to a pathogenic yeast form in mammalian hosts. In the laboratory, *H. capsulatum* grows in the yeast form at 37°C and in the filamentous form at room temperature. We previously identified four transcription factors, Ryp1-4, and showed that they are key regulators of a temperature-responsive genetic network that is required for yeast-phase growth in *H. capsulatum*. In this study, we performed immunoprecipitations using antibodies against Ryp2 and Ryp3, followed by mass spectrometry to identify Ryp2- and Ryp3-interacting proteins at 37°C. Among the diverse set of proteins that interact with Ryp2 and Ryp3, we characterized two Ryp2-interacting proteins with predicted response regulator domains. Response regulators and sensor histidine kinases form two-component regulatory systems that are often involved in sensing environmental signals using a phosphorelay mechanism. Because it was previously shown that a sensor histidine kinase, Drk1, is required for the yeast-phase growth (Nemecek et al 2006 *Science*), we hypothesized that the two Ryp2-interacting response regulators could also be important for yeast-phase growth in *H. capsulatum*. Confirming our hypothesis, knockdown of these two genes (named *RYP5* and *RYP6*) resulted in filamentous growth regardless of temperature, indicating that they are required for yeast-phase growth. Additionally, from our previous studies, we found that *RYP5* and *RYP6* are not regulated (directly or indirectly) by Ryp1-4, suggesting that Ryp5 and Ryp6 may act upstream of Ryp1-4. We are currently investigating whether Ryp5 and Ryp6 act together with Drk1 and upstream of Ryp1-4 to regulate yeast phase growth. These experiments are highly significant since they will reveal the importance of two-component regulatory systems for sensing host temperature and provide a molecular understanding of how a pathogenic fungus responds to host temperature to cause disease.

Modular and compartmentalized gene regulatory networks of *Fusarium graminearum*. Li Guo[1], Guoyi Zhao[2], Lixin Gao[2], Jin-Rong Xu[3], H. Corby Kistler[4], Li-Jun Ma[1]. 1) Department of Biochemistry and Molecular Biology, 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.

Fusarium head blight caused by filamentous ascomycete *Fusarium graminearum* (*Fg*) is a major limiting factor for global wheat production, leading to reduced yield and toxic grains containing trichothecene mycotoxins. Knowledge on the gene regulatory network (GRN) of this fungus is currently lacking but vital for understanding the pathobiology and developing effective disease management strategies. We have reconstructed an *Fg* GRN based on a collection of microarray data spanning a wide range of biological states using a Bayesian networks algorithm. The biological relevance of this *Fg* GRN is validated using prior biological knowledge of *Fg*. Our *Fg* GRN also partially overlaps with *Fg* Protein-Protein Interaction (FPPI) database, mainly covering protein complexes including a ribosomal complex and a spliceosome complex. The *Fg* GRN can be divided into eight distinct but interconnected modules that are enriched for different biological functions, such as cell cycle, protein synthesis and self-defense. These eight modules are differentially induced under different biological processes, such as pathogenesis, sexual development and conidia germination. Interestingly, we discover a distinct regulatory pattern for regulators from conserved and nonconserved genomic regions, suggesting that regulators may predominantly regulate target genes from the same genomic regions. Two regulatory modules have an overrepresentation of genes in nonconserved genomic regions. Such patterns indicates that *Fg* genome may have evolved with diverged regulatory circuits for the two genomic regions so that regulators and target genes from the same region combine to control specialized biological functions. This first ever reconstructed *Fg* GRN not only provides insight into the cell circuits of this pathogenic ascomycete, but also lays a foundation for future experimental studies on gene regulation controlling fundamental biological functions.

Genome-wide transcriptome analysis of cell wall remodeling in *Aspergillus niger* in response to the absence of galactofuranose biosynthesis. Joohae Park[1], Mark Arentshorst[1], Boris Tefsen[2], Ellen Lagendijk[1], Cees van den Hondel[1], Irma van Die[2], Arthur Ram[1]. 1) Molecular Microbiology and Biotechnology, Leiden University, Institute Biology Leiden, Leiden, The Netherlands; 2) Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands.

The biosynthesis of cell wall galactofuranose (Gal*f*) containing glycostructures such as galactomannan, *N*-glycans, *O*-glycans and glycosylinositolphosphoceramides in filamentous fungi are important to secure the integrity of the cell wall. A key gene in the biosynthesis of UDP-Galf is *ugmA* which encodes a UDP-galactopyranose mutase which is essential for the conversion of UDP-Gal*p* to UDP-Gal*f*. In A. *niger*, the absence of Gal*f* synthesis results in activation of the cell wall integrity (CWI)-pathway indicating that the Gal*f* biosynthesis is important for maintaining cell wall strength. To identify genes involved in maintaining cell wall integrity in response to the absence of galactofuranose biosynthesis, a genome-wide expression study was performed with the *ugmA* deletion strain. RNAseq analysis revealed 432 upregulated genes to be differentially expressed (Q-value <0.05) in the *ugmA* mutant compared to the wild-type and these genes encode enzymes involved in alpha-glucan synthesis (*agsA*), chitin synthesis (*gfaB, gnsA,* and *chsA*), beta-glucan remodeling (*bgxA, gelF,* and *dfgC*) and several (GPI)-anchored cell wall protein encoding genes. Interestingly, also the gene encoding the CWI-specific Map-kinase-kinase (*mkkA*) was induced in the *ugmA* mutant. *In silico* analysis of the 1-kb promoter regions of the differentially up-regulated genes in the *ugmA* mutant using an in house developed transcription factor binding site finder program, indicated overrepresentation of genes with RlmA or SteA binding sites. The importance of the RlmA and SteA transcription to induce cell wall remodelling genes is currently under investigation by constructing a *rlmA-ugmA* and *steA-ugmA* double mutants.

Understanding the Circadian Output Gene Regulatory Network using the Clock-Controlled Transcription Factor ADV-1 in *Neurospora crassa*. Oneida Ibarra[1], Rigzin Dekhang[1], Elham Aziz[2], Cheng Wu[1], Kristina Smith[3], Jill Emerson[4], Jay Dunlap[4], Michael Freitag[3], Matthew Sachs[1], James Galagan[2], Deborah Bell-Pedersen[1]. 1) Texas A&N University, 3258 Tamu, Department of Biology, College Station, TX; 2) Boston University, 44 Cummington St., Department of Biomedical Engineering, Boston, MA; 3) Oregon State University, 3021 Agriculture and Life Sciences Building, Center for Genome Research and Biocomputing, Corvallis, OR; 4) Dartmouth College, Remsen Room 702, Geisel School of Medicine at Dartmouth, Hanover, NH.

Organisms keep track of time, and are synchronized to the 24-hr environmental cycle, using a molecular circadian clock. About 30% of the genome is controlled by the clock at the level of transcript abundance in eukaryotic cells, but the mechanisms by which the clock regulates rhythms and peak phase in mRNA levels are not well understood. Using Neurospora as a model organism to investigate the circadian output gene network, we identified direct targets of the core clock component and blue light photoreceptor WHITE-COLLAR Complex (WCC) using chromatin immunoprecipitation, followed by sequencing (ChIP-seq). The direct targets of the WCC were enriched for 24 first-tier transcription factors (TFs), suggesting that aspects of light regulation and circadian output pathways are hierarchical. ADV-1, one of the first-tier TFs, is robustly rhythmic, defective in clock-controlled development, and is closely linked to the downstream developmental and metabolic network. In addition to the WCC, there are several first-tier TFs, including ADV-1 itself, that bind to the promoter of *adv-1*. ADV-1 in turn, binds to the promoters of downstream TF genes, and genes involved in metabolism, cell fusion, and development. Not surprisingly, many of the downstream targets of ADV-1 are clock-controlled, but they peak at different times of the day. Using TF deletion strains, we are testing the prediction that the upstream and downstream TF network revolving around ADV-1 generates distinct temporal dynamics of gene expression critical to the coordination of rhythmic processes, including rhythmic metabolism and development.

Thursday, March 19 3:00 PM–6:00 PM
Fred Farr Forum

Cytoskeleton, Endocytosis and Endosomes
Co-chairs: Norio Takeshita and Nick Read

Coupling of cisternal maturation and exocytic transport during Golgi exit involves all sorts of motors. Miguel Penalva[1], Mario Pinar[1], Victor García-Tagua[1], Miguel Hernández-González[1], Manuel Sánchez López-Berges[1], Herbert N. Arst[1,2], Xin Xiang[3], Areti Pantazopoulou[1]. 1) Cellular and Molecular Biology, CSIC Centro de Investigaciones Biológicas, Madrid, Spain; 2) Section of Microbiology, Imperial College London, Flowers Building, Armstrong Road, London SW7 2AZ, UK; 3) Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Few organisms have been as useful, and almost no genetic screen as influential, as the budding yeast and the Novick & Schekman genetic screen that uncovered the basic machinery of secretion in eukaryotes. Yet, 34 years later, our understanding of important aspects of secretion such as the molecular mechanisms that dictate traffic across, and exit from the Golgi is still wanting. A shortcoming of *S. cerevisiae* as experimental model is that it does not use microtubules (MTs) for intracellular traffic, whereas in filamentous fungi MTs and associated motors mediate long distance movement of endosomes and exocytic carriers. Hyphal cells of *Aspergillus nidulans* grow by apical extension at 0.5-1 µm/min at 37ºC, implying that exocytosis, which supplies materials to the growing tip, is highly active. Late Golgi cisternae undergo changes in composition to gradually lose Golgi identity while acquiring post-Golgi identity, consistent with the cisternal maturation model. Using GFP-RAB11 we have filmed how this maturation step precedes the journey of newborn post-Golgi carriers to the apex. These carriers, loaded with kinesin, dynein and myosin-5, move on a microtubule-based bidirectional conveyor belt relaying them to F-actin filaments, which mediate the final, apex-focusing step in exocytosis. Thus *Aspergillus nidulans* secretion is mechanistically similar to the transport of melanosomes in melanocyte dendrites.

Coordination of aminophospholipid asymmetry, P4 ATPases, and vesicle traffic during hyphal growth. Zachary Schultzhaus, Huijuan Yan, Brian Shaw. Plant Pathology and Microbiology, Texas A&M University, College Station, TX.

How proteins break symmetry and congregate in specific places inside cells is a major question in developmental biology, and fungal hyphae are excellent models for studying this phenomenon. Hyphae precisely localize a large group of proteins in order to colonize and maintain a constant shape. This organization includes a strict segregation of endocytosis and exocytosis into different areas of the apical compartment in growing hyphae. How endocytosis and exocytosis initialize, however, is unclear in these cells. Recently, the asymmetric nature of the phospholipid bilayer has attracted attention for playing roles in diverse processes such as phagocytosis and cell polarity. In this project we analyzed the role of phosphatidylserine distribution in fungal tip growth. The distribution of the phospholipid phosphatidylserine in growing hyphae was assessed. Phosphatidylserine concentrated on the outside of secretory vesicles, rather than the plasma membrane, in contrast to what is seen under normal conditions in budding yeast. Moreover, the deletion of phospholipid flippases caused a dispersal of phosphatidylerine secretory vesicles from the apex to the rest of the cytoplasm. These results indicate that phospholipid flippases (P4 ATPases) may be important for phosphatidylserine polarity on secretory vesicles, and thus for the localization of many tip proteins in growing cells.

Investigating the role of BAR domain proteins during plant infection by *Magnaporthe oryzae*. Magdalena Martin-Urdiroz, Martin J Egan, Miriam Oses-Ruiz, Darren Soanes, Nicholas J Talbot. School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, England, EX4 4QD, United Kingdom.

The blast fungus *Magnaporthe oryzae* causes a serious disease on a wide variety of grasses including rice. The infection process is

initiated when a spore lands on the surface of a leaf and attaches tightly to the cuticle. Then, the spore forms a polarized germ tube, which extends before swelling at its tip, changing direction and becoming flattened against the surface. This process constitutes a recognition phase, which precedes development of a specialized infection cell, the appressorium. This dome-shaped cell generates enormous turgor pressure, which is translated into physical force at its base to rupture the rice leaf cuticle using a narrow, rigid, penetration peg that invades plant tissue. In order to elaborate a penetration peg *M. oryzae* must regulate membrane curvature at the appressorium pore ahead of rapid cell wall biogenesis and apical growth. The generation of concave or convex curvature at membranes is thought to be induced by a variety of protein-driven mechanisms, including the action of BAR domain family proteins. In *M. oryzae*, seventeen BAR domain proteins have been identified. Atg24 (Snx4) and Snx41 are involved in mitophagy and pexophagy and endosomal sorting, respectively, but the specific function of the other BAR domain proteins is unknown. We are currently characterising homologs of Rvs167, Rvs161, KAR3, Cdc24, Snx4, Gvp36, Vps17, Vps5, Snx41, Rgd1, Rgd2, Bzz1, Hof1, Pil1, Lsp1 and SIP3. We have shown that Pil1 and Lsp1 constitute an eisosome, which we have shown is involved in endocytosis and plays roles in conidial germination and germ tube development. We have also found that Rvs167-GFP localizes to the centre of the appressorium pore prior to emergence of the penetration peg. *RVS167*, *ATG24* and *HOF1* are all differentially expressed during appressorium development, while expression of the genes *Lsp1*, *Pil1*, *Rgd1* and *Rgd2* is low. Here we report the localisation and functional analysis of BAR domain proteins in *M. oryzae* and, in particular, explore their role in septin-mediated appressorium penetration and the formation of penetration hyphae during plant infection.

Calcium signaling and cytoskeletal dynamics during hyphal growth and infection by the human fungal pathogen *Aspergillus fumigatus*. Constanze Seidel, Alberto Muñoz, Nick D. Read. Manchester Fungal Infection Group, University of Manchester, Manchester, United Kingdom.

The most common human mould infection is caused by *Aspergillus fumigatus*, and it has been estimated that more than 3 million people worldwide are infected with it. The capacity of *A. fumigatus* to respond appropriately to external signals underpins its saprotrophic and parasitic lifestyles, and calcium signaling plays a major role in this. Ca^{2+}-signaling and homeostasis are essential for the growth, differentiation and virulence of filamentous fungi although relatively little is known about the role of Ca^{2+} in these processes in filamentous fungi, and particularly in human fungal pathogens during invasive growth. We have developed techniques for the routine imaging of Ca^{2+}-dynamics in living *A. fumigatus* cells expressing the genetically encoded Ca^{2+}-sensor, GCaMP6s. Time-lapse-imaging of continuously growing hyphae of different filamentous fungi has shown that, instead of growing hyphae possessing a constant tip-focused gradient, transient pulsatile increases in Ca^{2+}-changes in hyphal tips occur with no discernible Ca^{2+}-gradient between pulses. Furthermore, we have demonstrated different Ca^{2+}-signatures in response to different types of environmental stress that might be encountered during infection. Cytoskeletal dynamics and motor proteins are also regulated by Ca^{2+}. To determine how the F-actin and the microtubule cytoskeleton are dynamically organized whilst growing over alveolar epithelial cell layers, we generated strains expressing α-tubulin and lifeact fused to GFP and tagRFP-T. These live-cell imaging studies have been combined with the use of pharmacological agents and mutant analyses to understand the roles of calcium signaling and the cytoskeleton during hyphal growth and infection.

Dynamics of the actin cytoskeleton in *Phytophthora infestans* hyphae and infection structures. Harold J.G. Meijer[1], Kiki Kots[1,2], Chenlei Hua[1], Tijs Ketelaar[2], Francine Govers[1]. 1) Lab. of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Lab. of Cell Biology, Wageningen University, Wageningen, Netherlands.

The actin cytoskeleton is a dynamic but well organized intracellular framework that is indispensable for the viability of eukaryotic cells. Its functions range from intracellular transport, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. We studied the actin cytoskeleton dynamics in the filamentous oomycete plant pathogen *Phytophthora infestans* in transgenic lines expressing the actin binding peptide Lifeact-eGFP by fluorescence microscopy. This showed that in hyphae actin filament cables and plaques are cortically localized. The distance between the hyphal tip and the first actin filament plaque correlated strongly with growth velocity. Upon growth termination, actin filament plaques appeared in the hyphal tip. The plaques were nearly immobile with average lifetimes exceeding one hour; much longer (over 500-fold) than the lifetimes of actin patches in fungi. Plaque assembly required ~30 seconds while disassembly took only ~10 seconds. In contrast to actin patches in yeast, plaque disassembly was not accompanied with formation and internalization of endocytic vesicles (Meijer et al. 2014, Cell. Microbiol.). We also investigated the *in vivo* actin dynamics during early stages of pathogenesis. At the site of contact with the plant cell a condensed transient actin structure was observed that resembles aster-like actin structures formed upon encountering hard surfaces. Our results suggest that the actin cytoskeleton has distinct functions during the *P. infestans* lifecycle. Future efforts will focus at identifying interactors and key regulators of the actin cytoskeleton and pinpoint features in the actin network that are unique for oomycetes.

Analysis of Septin Organization Using Polarized Fluorescence Microscopy. Molly McQuilken[1], Sara Abrahamsson[2], Shalin B. Mehta[3], Grant Harris[3], Amitabh Verma[3], Rudolf Oldenbourg[3], Amy S. Gladfelter[1]. 1) Biological Sciences, Dartmouth College, Hanover, NH; 2) Lulu and Anthony Wang Laboratory of Neural Circuits and Behavior, Rockefeller University, New York, NY 10065; 3) Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Septins are conserved filament-forming proteins that act in cytokinesis, membrane remodeling, cell polarization, and migration. They closely associate with membranes and help establish dramatic shapes in various fungi. Although septin function is critical for diverse cell events, it is not well understood how they assemble *in vivo* or how they are remodeled throughout the cell cycle. The orientation of the dipole moment of GFP has been well established, and thus, constraining GFP to an endogenous septin allows for an assessment of septin organization *in vivo* by polarization microscopy. Polarized fluorescence analysis has previously shown that septins filaments are paired in properly assembled higher order structures, and organized septin filaments undergo a coordinated 90° reorientation during cytokinesis *in vivo*. We developed a Multifocus Polarization Microscope (MF-PolScope) to evaluate septin organization in 3D through time to capture the assembly and rearrangement of higher-order septin structures, and analysis of mutant yeast strains with abnormally organized septins. MF-

PolScope imaging has enabled identification of septin interactors important for distinct aspects of the assembly, stability, and rearrangement of septins. One interactor necessary for proper assembly of septin higher order structure is the regulatory septin Shs1. Using the MF-PolScope we are not only able to show the organization of septin higher order structures, but we are able measure pairing of septin filaments; a measurement that is challenging in the wide-field PolScope system. We were able to identify disorganization of septin structure in Shs1 mutant lines is concomitant with an underlying lack of paired septin filaments. This work has provided more insight into the role of septins in fungal shaping.

The functional orthologue of the human tumor suppressor APC protein MigA plays a role in polarity determination in the filamentous fungus *Aspergillus nidulans*. Raphael Manck[1], Yuji Ishitsuka[2], Satur Herrero[1], Norio Takeshita[1], G. Ulrich Nienhaus[2], Reinhard Fischer[1]. 1) IAB - Microbiology, KIT, Karlsruhe, Germany; 2) CFN, KIT, Karlsruhe, Germany.

Polarity establishment and maintenance is an essential process conserved in all kingdoms and very obvious in filamentous fungi like *Aspergillus nidulans*. The cell needs an orchestrated polarization machinery to initiate and sustain a highly polarized structure such as a hypha. The microtubule (MT) and the actin cytoskeleton along with MT associated proteins such as MT plus-end tracking proteins (+TIP) play key roles in defining an internal polarity axis. In addition, MT's define the site of actin polymerization through the delivery of cell end marker proteins (1).

Here we describe MigA from *A. nidulans*, which is the first functional orthologue of the human tumor suppressor APC in filamentous fungi. APC is an essential regulator of radial glial polarity and construction of the cerebral cortex in mice (2). Furthermore it regulates axon arborization and cytoskeleton organization. MigA interacts with the membrane associated ApsA protein and is involved in spindle positioning during mitosis. Since MigA is related to yeast Kar9, this function is conserved in comparison to *Saccharomyces cerevisiae*. Moreover, MigA is also associated with septal and nuclear MT organizing centers and localizes in an EbA-dependent manner to assembling and retracting MT plus-ends. This characteristic classifies MigA as a +TIP. *A. nidulans* MigA forms a homodimer and is able to bind filamentous α-tubulin autonomously. In contrast to Kar9, MigA has another unexpected function. It is required for MT convergence and correct localization of the cell end markers TeaR and TeaA at the hyphal tip. MigA also interacts with the class V myosin MyoV. Taken together we propose an active Actin-MyoV-MigA-dependent guidance mechanism, the MigA-pathway, of MT's in the hyphal tip. Hence, actin and MT organization depend on each other.

(1) Fischer *et al.* (2008). Mol Microbiol. May; **68**(4):813-26

(2) Yokota *et al.* (2009). *Neuron*. Jan 15;**61**(1):42-56.

Dynamics of cytoplasmic microtubules: From experimental data to simulations with prediction potential. R. Gibeaux[1], S. Grava[2], A. Politi[1], C. Antony[1], F. Nedelec[1], P. Philippsen[2]. 1) EMBL Heidelberg, Germany; 2) Biozentrum, University of Basel, Switzerland.

Cytoplasmic microtubules (cMTs) of *Ashbya gossypii* exclusively emanate from the outer side of the spindle pole bodies, which are embedded in the nuclear envelope, and not from other sites in the multinucleated hyphae. This explains why cMTs in this fungus are not involved in vesicle transport, but solely act as cytoskeletal elements for pulling of nuclei. *A. gossypii* nuclei are very mobile: bidirectional movements prevent formation of nuclear aggregates, and bypassing of nuclei promotes nuclear mixing (Grava et al. Euk Cell 2011; Gibeaux et al. JCS 2012). The key molecular motor for these processes is dynein which is transported by a kinesin to the plus ends of cMTs and exerts pulling forces upon contact with the cortical anchor Num1. Mutants with shorter than wild-type cMTs lead to decreased nuclear mobility and mutants with longer than wild-type cMTs lead to increased bidirectional movements and bypassing of nuclei (Grava and Philippsen MBoC 2010). Despite these efforts it could not be conclusively demonstrated so far that growth dynamic of cMTs and pulling forces exerted by cortex-associated dynein are solely responsible for the observed nuclear movements. In addition, it is still unknown whether the high density of organelles visualized by electron tomography interferes with nuclear mobility or not (Gibeaux et al. Euk Cell 2013).

We therefore employed first principal modeling to quantitatively simulate the process. We first analyzed nuclear movements from live imaging data to derive quantitative measurements amenable to comparison with simulation data. Using parameters of cMT dynamics as measured in *A. gossypii*, we built a computational model that successfully and sufficiently reproduced *in vivo* behavior of nuclei in wild-type and in mutants with shorter or longer cMTs, respectively. We also tested the influence of both cytoplasmic stream and organelle crowding on nuclear movements.

Thursday, March 19 3:00 PM–6:00 PM
Kiln

Fungal Volatiles: Critical Signals for Fungal Interactions
Co-chairs: Joan Bennett and Seogchan Kang

Volatile Organic Compounds from *Trichoderma* Isolates: Their Impact on Plant Growth and Development. Samantha Y Lee, Richard Hung, Joan W. Bennett. Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Members of one of the most frequently isolated genera of free-living soil fungi, *Trichoderma*, are well studied for their ability to reduce plant disease, promote plant growth and productivity. Previously, we demonstrated that *T. viride* emitted VOCs induced growth promotion in the plant model system, *Arabidopsis thaliana*. The purpose of this study was to further examine and evaluate the effects of VOCs from several species of *Trichoderma* including: *T. aggressivum*, *T. asperellum*, *T. atroviride*, *T. brevicompactum*, *T. harzianum*, *T. longibrachiatum*, T. *pseudokoningii*, *T. virens* and *T. viride* (total of 20 strains) on *A. thaliana* and *Lycopersicum esculentum*. Plants and fungi were grown together while physically separated, allowing only gas exchanges to occur. Plants exposed to VOCs of several species of *Trichoderma* exhibited growth promotion and developmental changes including larger leaf size, increased shoot weight, increased lateral root branching, and increased total chlorophyll concentration. Plants exposed to VOCs emitted from *T. aggressivum*, *T. asperellum*, *T. pseudokoningii*, and *T. viride* had significant increases in total biomass and chlorophyll concentration. Interestingly, different strains of *T. asperellum* induced varied responses in plants and certain strains significantly inhibited plant growth. Real-Time qRT-PCR data shows that several auxin-related genes encoding efflux carrier, IAA, and SAUR-like proteins in *A. thaliana* were affected by the exposure to *Trichoderma* VOCs. Using CG-MS, we have identified over 100 unique compounds emitted by *Trichoderma*: hydrocarbons, sesquiterpenes, terpenes, straight-chain alkenes, saturated and unsaturated alcohols, aldehydes, ketones, aromatic compounds, and heterocyclics. Currently, we are studying the effects of individual VOCs on plant growth and conducting transcriptome study by using RNA-Seq and bioinformatics tools to understand volatile-mediated plant-fungus interactions.

Volatile compounds produced by plant-associated fungi play critical roles in plant growth and stress resistance. Ningxiao Li[1,2], Vasileios Bitas[2], Nate McCartney[3], Jung-Eun Kim[2], James Tumlinson[1,3], Seogchan Kang[1,2]. 1) Intercollege Graduate Degree Program in Plant Biology, the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA; 2) Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA; 3) Department of Entomology, The Pennsylvania State University, University Park, PA.

Because volatile compounds can travel far from the point of production through air, liquids, and porous soils, biogenic semio-volatile compounds can mediate both short- and long-distance organismal interactions. Animal- and plant-derived volatiles in directing animal behaviors (e.g. pheromones, homing cues to pollinators, parasitoids and biting insects) and volatile hormones, such as methyl jasmonate and methyl salicylate, as a language for plant-to-plant communication have been well-documented. Microbial volatiles also have been implicated as signals for mediating microbial interactions with other microbes, plants and animals. Accumulated evidence from plant growth promoting rhizobacteria suggested that certain microbial volatiles function as chemical effectors that manipulate plant physiology. However, the available knowledge on similar roles of fungal volatiles is very limited. *Fusarium oxysporum* and *Verticillium* spp. are common soil borne fungal species, with some strains causing vascular wilt diseases in many agriculturally important plants around the world. Given their economic importance, it is critical to better understand their ecology and pathology. Recent studies in our group showed that many isolates of *F. oxysporum* (47 out of 58) and all isolates from 10 *Verticillium* spp. significantly promoted plant growth via volatile production. Moreover, volatile-treated plants showed enhanced resistance to *Pseudomonas syringae* DC3000 and increased salt tolerance. Genetic and histochemical analyses indicated that fungal volatiles affect multiple phytohormone signaling pathways. Through targeted mutagenesis, we began uncovering the nature of fungal volatiles that affect plant growth and their biosynthetic pathways.

Oxylipin cross-talk between innate immunity and *Aspergillus fumigatus*. Gregory Fischer[1], Jun Yang[2], Chun-Jun Guo[3], Jonathan M. Palmer[4], Erwin Berthier[4], Taylor Dagenais[4], Xiaozhu Huang[5], Bruce D. Hammock[2], Clay C.C. Wang[3], Nancy P. Keller[1,4]. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Entomology and Comprehensive Cancer Center, University of California-Davis, Davis, CA; 3) Pharmacology and Pharmaceutical Sciences and Chemistry, University of Southern California, Los Angeles, CA; 4) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 5) UCSF Sandler Asthma Basic Research Center, University of California-San Francisco, San Francisco, CA.

Aspergillus fumigatus is a ubiquitous fungal pathogen causing diseases of the hyperimmune state (e.g. acute bronchopulmonary aspergillosis, ABPA) and the hypoimmune state (e.g. invasive aspergillosis, IA). We hypothesize oxylipin-producing oxygenases encoded within the *A. fumigatus* genome impact development of both diseases. *A. fumigatus* contains two lipoxygenases and three cyclooxygenase-like enzymes with considerable identity to human 5-lipoxygenase and cyclooxygenase enzymes, respectively. Through development of various oxygenase deletion and overexpression mutants, we have characterized an *A. fumigatus* oxylipin profile, detecting many oxylipins already known to exacerbate asthmatic symptoms when produced by a human host. Extracts containing oxylipins from a lipoxygenase overexpression strain (*OE::loxB*) led to hallmark traits of asthma including increased airway hyperresponsiveness, macrophage and eosinophil recruitment, and increased IgE levels in a murine model of asthma. Furthermore, disruption of a particular cyclooxygenase-like enzyme, PpoA, altered neutrophil recruitment, a primary mechanism by which hosts counter IA development. The acquisition of these oxygenase enzymes may present a novel, albeit unintentional, mechanism by which *A. fumigatus* exacerbates ABPA and/or IA development.

Influence of volatile organic compounds on *Fusarium graminearum* mycotoxin production. Martha Vaughan, Susan McCormick. USDA ARS, Peoria, IL.

Volatile organic compounds (VOCs) are involved in a diverse range of ecological interactions. Due to their low molecular weight, lipophilic nature, and high vapor pressure at ambient temperatures, they can serve as airborne signaling molecules that are capable of mediating inter and intraspecies communications. VOCs emitted by plants can directly contribute to the emitter's disease resistance, enhance the resistance of neighboring plants, and influence sporulation of colonizing fungal species. Correspondingly, fungal VOCs can alter the growth and metabolism of other fungal species and plants. Interestingly, production of a VOC, trichodiene, is the first step in the biosynthesis of Fusarium trichothecene mycotoxins. Despite the close association of this volatile with *in planta* trichothecene contaminants and the function of these mycotoxins as a virulence factor which enhances disease development, not much is known about the potential function of trichodiene as a volatile signal. Governed by the hypothesis that VOCs play a role in the interaction between wheat and *Fusarium graminearum*, the major causal agent of Fusarium head blight disease in wheat, we are investigating the influence of VOCs in mycotoxin production.

***Trichoderma* secondary metabolism and its relation to plant growth promotion.** Maria Fernanda Nieto Jacobo[1], Fatima Berenice Salazar-Badillo[1,2], Mark Braithwaite[1], Dianne Nguyen[1], Michael Rostas[1], Jorge Teodoro de Souza[1], Johanna Steyaert[1], Juan Francisco Jimenez-Bremont[2], Alison Stewart[1,3], Artemio Mendoza[1]. 1) Bio-Protection Reserch Centre, Lincoln University, Lincoln, Lincoln, New Zealand; 2) San Luis Potosi Institute of Scientific Research and Technology, San Luis Potosi, Mexico; 3) Marrone Bio Innovations, 2121 Second St, Davis, California 95618, USA.

Endophytic *Trichoderma* strains can improve plant growth, confer disease resistance and abiotic stress tolerance, and at the same time, obtain nutrients from their host plants without causing apparent disease symptoms. The continuation of a symbiotic relationship between endophytes and their respective hosts requires constant communication between the organisms. We have been investigating the role of the fungal-derived phytohormone indole acetic acid (IAA) and volatile organic compounds (VOCs) in the signalling dialogue between *Trichoderma* and the model plant *Arabidopsis thaliana*. A range of *Trichoderma* strains, selected for their ability to enhance root production in greenhouse experiments, were tested for IAA production in synthetic medium. Significant differences were observed in the ability to synthetize IAA between strains, and it was found to be medium dependent. Most of the *Trichoderma* strains induced anthocyanin production in the leaves, which may be an indication of stress. No significant differences were observed in most of *Trichoderma* strains in their abilities to induce *A. thaliana* root growth on Murashige & Skoog plates. In a separate bioassay conducted in gamma radiated soil, contrasting results were observed. While four strains significantly enhanced shoot weight, two had no effect on shoot weight and one strain negatively impacted total plant fitness, reducing growth by 50% compared to the untreated control. Thus, we suggest that although IAA could have an important role in the plant growth promotion, additional signals need to be considered in this process such as VOCs. Using a Petri dish split system, we demonstrated that VOCs produced by *Trichoderma* have an impact on plant fitness. The VOCs profiles of different strains will be discussed.

Non-trichothecene sesquiterpenes are produced by *Fusarium graminearum* PH-1, and are dependent on Tri5 expression. Chris Flynn[1], Karen Broz[2], Valeriu Bortnov[1], Claudia Schmidt-Dannert[1], H. Corby Kistler[2]. 1) University of Minnesota. Department of Biochemistry, Molecular Biology, and Biophysics. Saint Paul, MN, USA; 2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

Deoxynivalenol (DON) is the primary mycotoxin produced by the cereal disease causing ascomycete *F. graminearum*. DON is a trichothecene sesquiterpene derived via the trichodiene synthase (Tri5) product trichodiene. Sesquiterpenes, produced by sesquiterpene synthases (STS), are fifteen-carbon compounds made by many plants, bacteria, and fungi, often as defensive, pathogenesis-related, or signaling compounds. Tri5 is one of eight putative STSs in the *F. graminearum* genome. Here we describe Solid-Phase MicroExtraction (SPME) sampling of the volatile and soluble fractions of *F. graminearum* PH-1 shake flask cultures. When grown in trichothecene induction medium, PH-1 produces trichothecenes as expected, as well as several unanticipated non-trichothecene sesquiterpenes, whereas no volatile terpenes were detected when grown in non-inducing medium. Surprisingly, a *Δtri5* deletion strain grown in inducing conditions not only ceased production of the anticipated trichothecenes, but also did not produce the non-trichothecene sesquiterpenes. To test the possibility that *F. graminearum* Tri5 is a non-specific STS directly producing all observed sesquiterpenes, Tri5 was cloned from *F. graminearum* PH-1 cDNA, expressed in *E. coli*, and shown to produce primarily trichodiene. Therefore, while Tri5 expression in *F. graminearum* PH-1 is necessary for non-trichothecene sesquiterpene biosynthesis, direct catalysis by Tri5 is not sufficient to explain the diversity of sesquiterpenoids produced under trichothecene inducing conditions, nor can it explain the sesquiterpene deficient phenotype observed in the *Δtri5* strain. These findings suggest that the Tri5 expression, through an as-of-yet unidentified mechanism, is required for expression of non-trichothecene producing STSs under DON inducing conditions. While the role of trichothecenes in phytotoxicity is known, the biological function of non-trichothecene sesquiterpenes, specifically co-produced with trichothecenes, has not yet been determined.

Ammonia: a critical signal for fungal host interactions. Shiri Barad[1,2], Eduardo Espeso[3], Amir Sherman[4], Dov Prusky[1]. 1) Post Harvest Sci, Agricultural Res Org, Bet Dagan, Israel; 2) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 3) Department of Molecular and Cellular Biology, Centro de Investigaciones Biologicas (C.I.B.), Madrid, Spain; 4) Genomics Unit, ARO, The Volcani Center, Bet Dagan 50250, Israel.

Penicillium expansum, the causal agent of blue mold rot, causes severe postharvest fruit maceration through secretion of D-gluconic acid (GLA) and secondary metabolites such as the mycotoxin patulin in colonized tissue. While patulin was described to be produced by many *Penicillium* species, the conditions inducing patulin biosynthesis are not clear. Analysis of the pH modulating factors produced by *P. expansum* detected that beside the production of GLA, the fungus can accumulate ammonia, both in culture and in vivo, at the leading edge

of the colonized tissue. Treatment with exogenic ammonia to growing *P. expansum* on solid media induced patulin biosynthesis by inducing the expression of the global pH regulator and the secondary-metabolism-regulating transcription factors *pacC* and *laeA*, respectively. Experiments, by which ammonia was modulated by growth under limited or excessive carbon source, showed that ammonia is an efficient inducer of the expression of intermediate factors modulating patulin accumulation, including *pacC, laeA* and *idh* (one of the last genes in patulin biosynthesis), at pH ranges of 3.7-4.0 in the presence of GLA. Analysis of relative expression of *pacC* at acidic buffered conditions indicated that ammonia may induce a 2 fold increase in the relative expression of *pacC* at pH 4.5, probably as a result of internal fungal alkalization. Present result indicates that conditions enhancing ammonia accumulation by colonizing *P. expansum* may activate patulin accumulation and several pathogenicity factors which contribute to host-tissue colonization by *P. expansum* under acidic conditions.

Hydroxy Fatty Acids Sensing and Surface Perception by *Ustilago maydis*. Pierre Grognet, Regine Kahmann. Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

For *Ustilago maydis*, filamentous growth and appressoria formation are the key stages for infection. In nature, the switch from yeast to hyphae takes place on the plant surface in response to sensing plant signals. It is known that two different stimuli are sensed: hydrophobicity and hydroxy-fatty acids (HFAs). While two membrane proteins involved in hydrophobicity sensing are known (Msb2 and Sho1), nothing is known yet about the receptors involved in HFAs sensing. HFAs trigger filamentation of *U. maydis* and are required for efficient appressoria formation on a hydrophobic surface. HFAs belong to a broader family of compounds called oxylipins. These molecules are present in all major groups of eukaryotes and play crucial roles in communication either between individuals or as internal communication signal. All known receptors for oxylipins are G protein-coupled receptors (GPCR). Mining the genome of *U. maydis* allowed us to identify 63 putative 7 trans-membrane domains proteins and spot candidate oxylipins receptors. Here we discuss the role of these candidate receptors in HFA sensing and provide insight into the downstream signaling pathways triggered by HFAs.

Thursday, March 19 3:00 PM–6:00 PM
Heather

Molecular Evolution of Antifungal Resistance
Co-chairs: Richard Oliver and Paul Bowyer

Fungal cell wall remodelling and antifungal drug tolerance. Carol Munro, Louise Walker, Keunsook Lee, Sami Alawfi, Donna MacCallum, Neil Gow. School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

The *Candida albicans* cell wall is a dynamic organelle, primarily composed of chitin, β-1,3-glucan, β-1,6-glucan and mannoproteins. The echinocandin antifungal drugs target the cell wall by inhibiting β-1,3-glucan synthesis. The echinocandins generally provide effective therapy but sporadic breakthrough infections caused by resistant *Candida* isolates have been reported. *C. albicans* acquires echinocandin resistance through point mutations in the *FKS* target genes. In addition *C. albicans* responds to sub-MIC echinocandins by up-regulating chitin biosynthesis through activation of cell wall salvage pathways, that involve PKC and calcium/calcineurin signalling. Cells with elevated chitin are less susceptible to echinocandins *in vitro*, as well as in infection models. Other *Candida* species (with the exception of *Candida glabrata*) and *Aspergillus fumigatus* also activate compensatory chitin production when challenged with sub-MIC echinocandins, resulting in drug tolerance.

Substantial changes in the cell wall glycoproteome are also triggered by cell wall and envelope stresses, including echinocandin treatment. Several predicted carbohydrate-active enzymes (CAZy) enzymes involved in modulating and cross-linking chitin and glucan (Phr1, Phr2, Pga4, Crh11, Utr2) are among the cell wall proteins (CWPs) positively regulated in response to cell wall damage. The PKC cell wall integrity pathway MAP kinase, Mkc1, and the transcription factor, Rlm1, are important in the regulation of surface expression of these CWPs. Overexpression or deletion of specific CWP genes results in altered caspofungin susceptibility, paradoxical growth and host-pathogen interactions.

RNA-seq analysis of *Cercospora beticola* DMI-resistant and -sensitive strains in response to tetraconazole. Melvin Bolton[1], Luigi Faino[2], Bart Thomma[2], Gary Secor[3]. 1) USDA - ARS, Fargo, ND, USA; 2) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 3) 3Department of Plant Pathology, North Dakota State University, Fargo, ND, USA.

The hemi-biotrophic fungus *Cercospora beticola* causes Cercospora leaf spot (CLS) of sugarbeet. CLS management measures rely on the application of sterol demethylation inhibitor (DMI) fungicides. Reduced sensitivity to DMIs has been reported recently in sugarbeet growing regions worldwide. We have shown previously that *CbCyp51*, which encodes the DMI target enzyme sterol P450 14α-demethylase in *C. beticola*, is over-expressed in DMI-resistant isolates. After exposure to tetraconazole, DMI-resistant isolates respond with further induction of *CbCyp51*. However, no *CbCyp51* promoter or gene mutations were associated with DMI resistance. To gain additional insight and identify other mechanisms involved with DMI-resistance, we used RNA-seq to identify genes differentially expressed in DMI-resistant and -sensitive *C. beticola* isolates upon exposure to tetraconazole or the control treatment *in vitro*. Interestingly, both the resistant and sensitive isolates responded with a marked induction of 15 of the 22 genes in the ergosterol biosynthesis pathway in response to tetraconazole. All 15 genes were induced to similar expression levels between the two isolates except for *CbCyp51* and *CbErg3*, which were ~16- and 2-fold higher in the resistant isolate when compared to the sensitive isolate, respectively. In total, 110 genes were uniquely differentially-expressed in the DMI-resistant isolate. Genes previously implicated with

fungicide resistance such as ABC and MFS transporters were identified. The most highly induced gene in the DMI-resistant isolate encodes a protein with multiple transmembrane regions known to provide DMI resistance in yeast. This protein is not known to provide efflux activity, but may bind directly to the fungicide. Further characterization of this gene and a detailed pathway analysis will be presented.

Accumulation of mutations in *Blumeria graminis* f. sp. *hordei* CYP51 confers positive and negative cross-resistance to triazole fungicides. Madeline Tucker[1], Francisco Lopez Ruiz[1], Hans Cools[2], Jonathan Mullins[3], Richard Oliver[1]. 1) Environment and Agriculture, Curtin University, Perth, Western Australia 6102, Australia; 2) Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; 3) Institute of Life Science and College of Medicine, Swansea University, Swansea, Wales SA2 8PP, United Kingdom.

Powdery mildews are amongst the most pervasive diseases worldwide. The effects of powdery mildews on crop yields vary widely with the phenology of the crop and its husbandry. Loses in cereals can amount to 40% in harvested grain while fruit infections in other crops like grapes can result in complete loss of the crop. Here we report the discovery of new mutations in the target site of the demethylation inhibitor (DMI) fungicides (*Cyp51* gene) in the barley powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*) and dissect the contribution of each mutation to the final resistant phenotype. Sequencing of the *Bgh Cyp51* target in a subset of isolates showing reduced triazole sensitivity, established a clear correlation with amino acid alterations Y136F, K171E, M301I, R327G and S509T in the *Bgh* CYP51 variants. Characterization of the mutations in a *Saccharomyces cerevisiae* mutant carrying a doxycycline-regulatable promoter controlling native *Cyp51* expression revealed that yeast genotypes harbouring the S509T mutation were less sensitive to all triazoles except fluquinconazole, which was significantly more effective. Protein structural modelling showed a strong correlation between fungicide sensitivity and the heme cavity volume. Interestingly, fluquinconazole docking studies indicated a potential negative cross resistance effect as a result of the interaction established between the proteins carrying the S509T mutation and this azole. The in silico appraisal of binding efficiencies between specific triazoles and CYP51 variants promises to facilitate the design of resistance management strategies that counterselect the most abundant mutations and considerably extend the useful life of this critical group of fungicides. The implications of these findings for powdery mildew fungicide resistance management in Australia are discussed.

A regular unicellular stage facilitates adaptation: the emergence of azole resistance in *Aspergillus fumigatus* . Jianhua Zhang[1], Alfons Debets[1], Paul Verweij[2], Willem Melchers[2], Bas Zwaan[1], Sijmen Schoustra[1]. 1) Laboratory of Genetics, Wageningen University, Wageningen, Netherlands; 2) Department of Medical Microbiology, Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands.

Understanding the occurrence and spread of azole resistance in *Aspergillus fumigatus* is crucial for public health. It has been hypothesized that a unicellular stage via asexual sporulation is essential for phenotypic expression of azole resistance mutations in *A. fumigatus* facilitating subsequent spread through natural selection. We assessed the advantage of unicellular stage via asexual sporulation by growing the fungus under pressure of one of five different azole fungicides and monitoring the rate of adaptation between scenarios of, (i) exclusive mycelium growth (multicellular stage) without asexual sporulation, and, (ii) growth allowing asexual sporulation (unicellular stage). Our results unequivocally show that unicellular stage via asexual sporulation enhances adaptation over a fixed period of evolutionary time. This can be explained by the combination of more effective selection because of the transition from a multicellular to a unicellular stage, and by increased mutation supply due to the sporulation process, which involves numerous mitotic divisions. Our insights are essential to unravel the fungal adaptation strategies are highly relevant for resistance development in the natural environment, but also for patients with chronic aspergillus diseases. Uncovering the pathways to adaptation will help to improve our current management strategies both in agricultural and medical fields.

Key words: *Aspergillus fumigatus*; azole resistance; unicellular and multicellular; asexual sporulation; experimental evolution; MIC value; mycelial growth rate.

Breaking the mould - drug resistance in fungal disease. Paul Bowyer. Manchester Fungal Infection Group, Faculty of Medicine and Human Science, University , manchester, United Kingdom.

Fungi are ubiquitous and human exposure to fungi is inescapable. Global mortality due to fungal disease rivals that caused by malaria and tuberculosis. Millions more suffer chronic allergic disease associated with fungal colonisation or exposure. Currently azoles are the main therapeutic option for fungal disease and remain the only oral therapy. Resistance to azoles is rising rapidly in certain countries.

Here we survey the prevalence and nature of azole resistance across the world. The first cases of azole resistance in clinically important fungi were documented over 20 years ago. Since then the frequency of resistance has increased rapidly although there are dramatic geographical differences in nature and prevalence.

Most known mechanisms of resistance involve mutation of the gene encoding the target protein or up – regulation of drug efflux however such mechanisms are unable to account for a large percentage of observed clinical drug resistance. Recent work in our laboratory and others across the world has elucidated several new mechanisms of resistance that not only display clinical relevance but also shed light on the mechanism of drug action.

Succinate-dehydrogenase inhibitors (SDHIs) resistance evolution in cereal pathogens. Stefano Torriani, Regula Frey, Jürg Wullschleger, Carolina Buitrago, Stephane Bieri, Gabriel Scalliet, Helge Sierotzki. Syngenta, Stein, Switzerland.

Disease control is critical to cereal production in Europe; several commonly occurring diseases pose significant threat to yields and grain quality. In high disease pressure year, yield loss can be significant. Nowadays, cereal pathogens are mainly controlled by quinone outside inhibitors (QoIs), succinate-dehydrogenase inhibitors (SDHIs), demethylation inhibitors (DMIs) and multi-site fungicides, such as chlorotalonil. Resistances have been reported to the major fungicide classes. Therefore, understanding of evolutionary processes responsible for emergence and spread of resistance coupled to long term monitoring are essential to infer adequate anti-resistance management strategies. SDHIs block the TCA cycle at the level of succinate to fumarate oxidation, leading to an inhibition of respiration. SDHI resistance has been observed in 14 fungal pathogens to date and is caused by different mutations in the target genes encoding for the mitochondrial succinate dehydrogenase (SDH) enzyme. The SDH enzyme is composed by four subunits encoded by distinct genes (sdh-A to D). Mutations in sdh-B to D associate in different cereal pathogens to decreased SDHI sensitivity. Recently, *Zymoseptoria tritici* isolates carrying mutations T79N and W80S in sdh-C and N225T in sdh-B were reported. These mutations confer only limited resistance level. In *Pyrenophora teres* the first resistance allele H277Y in sdh-B was reported in 2012. In 2013 mutation associated to decreased sensitivity were found in all three SDH subunits (sdh-C G79R was the most frequent). In this study we describe the evolutionary forces shaping SDHI resistance in field and we will discuss the evolution of single target genes and how their combination into a functional enzyme might shape the response to fungicide.

DMI resistance in *Zymoseptoria tritici*: a history of gradual molecular evolution. Patrick Brunner, Bruce McDonald. Integrative Biology Zurich, ETH Zurich, Zurich, Switzerland.

Fungicide resistance in crop pathogens is a global threat to food production but surprisingly little is known about the evolutionary processes associated with the emergence and spread of fungicide resistance. We evaluated early, mid and late stages in the evolution of fungicide resistance using the wheat pathogen *Zymoseptoria tritici*, taking advantage of a global isolate collection spanning +20 years. We analyzed sequences of the nuclear *CYP51* gene implicated in multiple-mutation resistance to azole fungicides. Results are discussed with particular regard to the roles of selection and intragenic recombination as driving evolutionary forces.

Genetic analysis of multi-drug-resistance (MDR) in *Mycosphaerella graminicola (Zymoseptoria tritici)* field isolates. Selim Omrane[1], Colette Audéon[1], Amandine Ignace[1], Clémentine Duplaix[1], Hind Sghyer[1], Lamia Aouini[2], Gert Kema[2], Anne-Sophie Walker[2], Sabine Fillinger[2]. 1) INRA, AgroParisTech, UMR BIOGER, Avenue Lucien Brétignières, F78850 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*, but also plant pathogenic fungi, e.g., *Botrytis cinerea*, *Oculimacula yallundae* and *Mycosphaerella graminicola* are concerned by this phenomenon.

MDR strains were detected in septoria leaf blotch (*M. graminicola*) field populations since 2008. These strains are cross-resistant to fungicides with different modes of action due to active fungicide efflux. In a previous study, we identified the *MgMFS1* gene overexpressed in all tested MDR field strains (Omrane et al., 2015). This gene encodes a major facilitator membrane transporter whose inactivation abolished the MDR phenotype in at least one field strain.

We went out to identify the mutation(s) responsible for MDR phenotype in two isolated strains (MDR6 and MDR7). Crosses between both MDR strains showed that *mdr6* and *mdr7 loci* are closely linked. A bulk-segregant analysis coupled to next generation sequencing showed a clear co-segregation between phenotypes and the left arm of chromosome 7. This region harbors 14 genes including the gene. We identified a 519 bp insert (LTR-like) in both MDR strains as well as in other (but not all) MDR field strains. Genotyping of the progenies for the promoter insert showed a clear, but not exclusive correlation between the *MgMFS1* promoter insert and the MDR phenotype. These results indicate that the LTR-like insert is responsible for the MDR phenotype, potentially *via MgMFS1* overexpression, but also that an additional and independent mutation confers the MDR phenotype to strain MDR6.

Thursday, March 19 3:00 PM–6:00 PM
Nautilus

Circadian Rhythms and Photobiology
Co-chairs: Deborah Bell-Pedersen and Christian Hong

Light-responsive transcription factors (LTFs) regulate differentiation and virulence in the gray mold fungus *Botrytis cinerea*. Kim Cohrs, Julia Schumacher. IBBP, WWU Münster, Schlossplatz 8, 48143 Münster, Germany.

Botrytis cinerea is the causal agent of gray mold diseases in a range of dicotyledonous plant species. The fungus reproduces asexually by forming macroconidia for dispersal and sclerotia for survival; the latter also participate in sexual reproduction by bearing the apothecia after fertilization by microconidia. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. The fungus responds to different wavelengths of light i.e. to near-UV, blue, red and far-red light suggesting that the eleven photoreceptors (two cryptochromes, four LOV proteins, two opsins, three phytochromes) are involved in light perception and initiation of downstream signaling events. Microarray analyses revealed 293 light-responsive genes including six TF-encoding (LTFs) and seven photoreceptor-encoding genes. The White Collar complex (WCC) formed by two GATA-type TFs is crucial for the response to blue/ white

light as it induces the transcriptional response of many genes (Canessa et al. 2013). However, light induction of some genes still occurs in its absence and the complex also works as repressor of light-induced genes indicating the contribution of other wavelengths/ photoreceptors to drive white light-responsive gene expression. LTFs are considered regulators of differentiation in *B. cinerea* by either promoting conidiation and/ or repressing sclerotial development. In fact, functional characterization of BcLTF1 - 3 (orthologs of *Neurospora crassa* SUB-1, SAH-1, and CSP-1) confirmed their involvement. Thus, BcLTF1 functions as repressor of conidiation and is furthermore involved in virulence, oxidative stress response and secondary metabolism (Schumacher et al. 2014). BcLTF2 appears to be the master regulator of conidiation in this system as its overexpression is sufficient to induce conidiation while its deletion abolishes conidiation. BcLTF3 is required for the differentiation of the conidia; its deletion results in malformed conidiophores that form sterile hyphae instead of conidia. As mutations of the LTFs affect the expression levels of other LTF-encoding genes, we assume a comprehensive network of TFs and photoreceptors that regulates light responses in *B. cinerea*.

Functions of ENVOY in *Trichoderma reesei*. Monika Schmoll[1], Jameela Lokhandwala[2,3], Hilary H. Hopkins[2,3], Aroa Rodriguez-Iglesias[1], Christoph Dattenboeck[1], Brian D. Zoltowski[2,3]. 1) Department Health and Environment, Bioresources, Austrian Institute of Technology AIT, Tulln, Austria; 2) Department of Chemistry, Southern Methodist University, Dallas, TX 75275, USA; 3) Center for Drug Discovery, Design and Delivery at Dedman College, Southern Methodist University.

Trichoderma reesei is one of the most prolific producers of plant cell wall degrading enzymes. In recent years, we showed that carbon metabolism including cellulase gene regulation are targets of the light signaling pathway. ENV1 (ENVOY) is one of the components involved in this regulation. Thereby, ENV1 plays important roles in cellulase regulation and carbon utilization on many carbon sources predominantly in light, but also has functions in darkness. One link between light signaling and nutrient signaling is established by regulatory interaction between ENV1 and the phosducin like protein PhLP1. Additionally, ENV1 is involved in regulation of sexual and asexual development. Nevertheless, despite similar output pathways of ENV1 and *Neurospora crassa* VVD, significant differences were found in their effects on gene regulation and development.

Analysis of the crystal structure of different variants of ENV1 indicated a function of C96 in oxidative stress response. Indeed this amino acid was found to be important for response to oxidative stress in vivo and moreover it was relevant for cellulase regulation. Also for T101, which is responsible for a different photocycle lifetime of ENV1 compared to VVD, a function in stress response was observed. Development was not affected by alteration of these two amino acids. In both cases, the presence of the respective amino acid appears to be evolutionary conserved across genera related to *T. reesei* vs. *N. crassa*.

Length of the photocycle in the VVD photoreceptor controls photo-adaptation and a dynamic VVD-WWC pool. Jennifer Loros[1], Arko Dasgupta[2], Chen Hui Chen[3], Changhwan Lee[4], Amy Gladfelter[5], Jay Dunlap[2]. 1) Dept Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Dept Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH; 3) Duke University Medical Center, Durham, NC; 4) University of Wisconsin,Madison, WI; 5) Dept Biological Sciences, Dartmouth College, Hanover, NH.

Most organisms on earth sense light through the use of chromophore bearing photoreceptive proteins with distinct and characteristic photocycle lengths, yet the biological significance of photocycle length is neither understood nor been tested. In the filamentous fungus Neurospora crassa VIVID (VVD) is a critical player in the process of photoadaptation, the attenuation of light induced responses and the ability to maintain photosensitivity in response to changing light intensities. Detailed in vitro analysis of the photochemistry of the blue light sensing, FAD binding, LOV domain of VVD has previously revealed residues around the site of photo-adduct formation that influence the stability of the adduct state, altering the photocycle length. We have examined the biological significance of VVD photocycle length to photoadaptation and report that a double substitution mutant (vvdI74V&I85V), previously shown to have a very fast light to dark state reversion in vitro, shows significantly reduced interaction with the White Collar Complex (WCC) resulting in a substantial photoadaptation defect. This reduced interaction impacts WHITE COLLAR-1 (WC-1) protein stability when N. crassa is exposed to constant light with the result that mutant VVD is unable to form a dynamic VVD-WCC pool of the size required for photoadaptation as assayed both by attenuation of gene expression and the ability to respond to increasing light intensity. Additionally, transcription of the clock gene frequency (frq) is sensitive to changing light intensity in a wild-type strain but not in the fast photo-reversion mutant indicating that the establishment of this dynamic VVD-WCC pool is essential in general light and circadian biology.

Characterization of light and circadian regulation in the necrotrophic fungus *Botrytis cinerea* and its role in pathogenesis using *Arabidopsis thaliana* as a plant model. M. Hevia, P. Canessa, H. Muller, L. F. Larrondo. Millennium Nucleus for Fungal Integrative and Synthetic Biology and Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

The necrotrophic fungus *Botrytis cinerea* is a phytopathogen that infects over 200 different plant species, ranking as the second most important plant pathogenic fungus based on its scientific-economic relevance. In order to understand how environmental signals affect is pathogenic potential, we have been evaluating the effect of light and circadian regulation on this fungus. Thus, we have identified that some, but not all light responses, are processed by a LOV domain transcription factor, which is the orthologue of *Neurospora crassa* WC-1. Thus, light modulates pathogenicity, and a strain that is devoid of BcWC-1 generates smaller lesions in the light, but not in the dark (where it behaves like the WT). We have also started the characterization of the *B. cinerea* circadian clock, which is composed of a FRQ protein orthologue and a transcriptional complex formed by WC-1 and WC-2. Our results indicate that *bcfrq* mRNA presents daily oscillations in a light-dark cycle and in constant darkness (DD), rhythms which are lost in a *bcwc-1 KO* strain. We have observed oscillatory levels of the BcFRQ protein under temperature cycles and DD. Both the *bcfrq* mRNA and BcFRQ protein anticipate cyclical-environmental changes (light or temperature), a key characteristic of circadian behavior. Importantly, we have observed an impaired infection process using *bcfrq* and *bcwc-1 KO* strains. Moreover, we demonstrate that the outcome of the plant- fungal pathogen interaction

using *Arabidopsis thaliana* and *B. cinerea* as working models varies with the time of day. Altogether, these results provide the first evidence indicating the existence and importance of a circadian clock in a pathogenic fungus, putting forward the concept that fungal clocks can synchronize key elements of virulence and pathogenesis. Fondecyt 1131030, MN-FISB NC120043.

Two circadian oscillators function to coordinately regulate circadian rhythmicity in *Neurospora crassa*. Nirmala Karunarathna[1], Renato de Paula[1], Bin Wang[2], Jay Dunlap[2], Deborah Bell-Pedersen[2]. 1) 3258 TAMU, Biological Sciences Building West, College Station, TX; 2) Dartmouth Medical School, Hanover, NH.

Most eukaryotes possess a circadian timing mechanism composed of at least a core circadian oscillator that uses proteins with opposing functions to form a negative feedback loop: positive-acting elements stimulate expression of the negative-acting elements, and the negative elements physically interact with the positive elements to inhibit their function. While the molecular details of the core oscillator are well described, new data suggests that the clock is comprised of multiple oscillators. For example, rhythmic behavior has been observed in canonical clock mutants of Neurospora, Drosophila, and mammals. In Neurospora, oscillations that are unmasked when the core circadian oscillator, the FRQ/WC oscillator (FWO), is eliminated have been referred to as FRQ-less oscillations that are driven by FRQ-less oscillators (FLOs).We recently discovered a set of genes, including *ccg-16* of unknown function, which are rhythmic in constant dark in strains that lack FRQ, and thus a functional FWO, but which require the WCC for rhythmicity. In addition, mRNA and protein levels of *ccg-16* cycle in constant light, conditions in which the FWO is arrhythmic. These data indicated the existence of a second oscillator in Neurospora cells, called the WCC-FRQ-less oscillator (WC-FLO), which may be coupled to the FWO through the WCC. We hypothesized that components of the WC-FLO physically interact with the WCC, similar to the interaction of FRQ with the WCC required negative feedback of the FWO. To test this hypothesis, knockouts of genes encoding WCC interacting proteins were examined for loss of *ccg-16:luciferase* reporter rhythms. Deletion of a WCC-interacting and clock-controlled transcription factor abolished *ccg-16* rhythms, suggesting a role for this protein in the WC-FLO mechanism, or in an output pathway from the WCC-FLO. Experiments are in progress to distinguish between these possibilities.

A HAD family phosphatase PSR-1 regulates Circadian Output pathway in *Neurospora crassa*. X. Zhou, B. Wang, C. Mallappa, J. Loros, J. Dunlap. Department of Genetics and Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH03755, USA.

Circadian clocks are ubiquitous in eukaryotic organisms where they are used to anticipate regularly occurring diurnal and seasonal environmental changes. *Neurospora crassa* has been used as a principal model organism for studying the circadian system for more than half a century. Nevertheless, little is known regarding pathways connecting the core clock to its output pathways. Here, we demonstrate a HAD family phosphatase PSR-1 is involved in circadian clock output. The *psr-1* deletion mutant has a circadian output defect on race tubes under free running conditions; constant conidiation is observed instead of rhythmic banding. However, further analysis indicates that the FRQ-WCC (FREQUENCY-WHITE COLLAR COMPLEX) oscillator functions normally in *Δpsr*-1 strains although with a three-hour phase delay. PSR-1 is important for maintaining WC-1 (WHITE COLLAR-1) protein and phosphorylation levels and for the interaction between VVD (VIVID) and WC-1. Increased WC-1 protein amounts can partially rescue the phase delay phenotype seen in the *Δpsr-1* mutant. Protein purification of PSR-1 shows it is part of a PSR-1/WHI-2 complex and that *Δ*whi-*2* has phenotypes similar to those observed for *Δpsr-1*. Together, our findings suggest that the PSR-1/WHI-2 complex participates in *Neurospora* clock function by regulating WC-1 levels.

Interconnected network of circadian rhythms and DNA damage response. Judit Zamborszky, Toru Matsu-ura, Jaesang Kwon, Mokryun Baek, Christian Hong. Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH.

The maintenance of genome integrity is essential for organisms. Recent findings indicate bidirectional influence between DNA damage response (DDR) and circadian rhythms. In both *Neurospora crassa* and *Mus musculus*, activated DNA damage response checkpoint kinase, PRD-4 (CHK2), phosphorylates a core clock components (i.e. FRQ in Neurospora and PER1 in mouse) and induces phase advances of circadian rhythms. On the other hand, circadian rhythms modulate ATR-mediated DNA damage response. However, detailed understanding of this network between circadian rhythms and DNA damage response remain elusive. In this report, we demonstrate circadian gene expression of key DDR components, *mus-21* (ATM) and *prd-4* (CHK2) in *Neurospora crassa*. These oscillations are abolished in circadian arrhythmic mutant, frq^{ko}. More importantly, rhythmic expression of *mus-21* and *prd-4* result in distinct circadian time-dependent DNA damage responses, which transiently disrupts conidiation-banding patterns upon DNA damage. Our findings unravel optimized circadian clock-dependent operations of DNA damage response mechanisms via *mus-21* and *prd-4*.

A natural light-inducible transcription system to characterize transcription dynamics. Francois Cesbron, Michael Oehler, Nati Ha, Gencer Sancar, Michael Brunner. University of Heidelberg Biochemistry Center Im Neuenheimer Feld 328 D-69120 Heidelberg Germany.

Genes are often transcribed in random bursts followed by inactive periods during which promoters are refractory towards restimulation. We employed the light-activatable White Collar Complex of *Neurospora* to study transcriptional bursting in a population approach. Activation of the WCC by a light pulse triggers a synchronized wave of transcription from the *frequency* promoter followed by an extended period of ~1 h during which the promoter is refractory towards restimulation. When challenged by a second light pulse, the newly activated WCC binds to refractory promoters and has the potential to recruit RNA polymerase II. However, accumulation of Pol II and phosphorylation of its C-terminal domain repeats at serine 5 is impaired. The data suggests that refractory promoters carry a physical memory of their previous history. Genome-wide analysis of light-induced transcription suggests that refractoriness is rather widespread and a property of promoter architecture.

Thursday, March 19 3:00 PM–6:00 PM
Scripps

Education and Outreach
Co-chairs: Marilee Ramesh and Lynne Boddy

Integrating strategic partnerships, education, and outreach to foster a research culture at a predominantly undergraduate institution. J.E. Flaherty[1], B.H. Bluhm[2], A.M. Fakhoury[3], J-R. Xu[4]. 1) Science and Mathematics, Coker College, Hartsville, SC; 2) Plant Pathology, University of Arkansas, Fayetteville, AR; 3) Plant Soil and Agricultural Systems, Southern Illinois University, Carbondale, IL; 4) Botany and Plant Pathology, Purdue University, West Lafayette, IN.

According to the Gallup-Purdue Index Report (2014), two notable factors that were found to contribute to a college graduate's long-term success relate to positive engagement with an encouraging mentor and working on a project that took a semester or more to complete. Undergraduate research provides a clear path for a student to have experienced these and other elements of experiential and deep learning. This presentation will focus on how our vision to transform conventional thinking about the roles undergraduates play in the process of discovery led to the establishment of a highly collaborative research program in fungal genetics built on undergraduate participation. Specifically, students at Coker College seek to discover and characterize genes involved in fungal development. Coker College is a private, unaffiliated liberal-arts college with a student body of nearly 1,200 students. Coker students have conducted research in collaborators' labs at the University of Arkansas, Southern Illinois University, and Purdue University. Additionally, multiple fungal genomics projects (re-sequencing mutant genomes and RNAseq experiments performed at the MUSC ProteoGenomics facility) have served to provide valuable experiences for students, strengthen existing relationships, and further enhance the research capacity at Coker College. This presentation will also describe outreach programs (*e.g.*, "diSCovering Science"), summer workshops, and classes developed, to serve K-12 students and teachers in the broader community.

A novel role for an SR/RRM family mRNA shuttling binding protein in cell cycle regulation in *Aspergillus nidulans*. S. L. Anglin[1], S. James[2]. 1) Dept. Biology, Millsaps College, Jackson, MS; 2) Dept. Biology, Gettysburg College, Gettysburg, PA.

SR/RRM proteins are a class of small nuclear ribonucleoproteins known to have functions in spliceosome assembly and catalysis as well as in mRNA transcription and export. Heretofore, this family of proteins has not been implicated in the regulation of cell division. We recently reported that the *Aspergillus nidulans snxA* gene encodes an ortholog of budding yeast Hrb1/Gbp2 and of human hnRNP-M, members of the SR/RRM protein family. hnRNP-M is a ubiquitous and highly expressed nuclear protein in human tissues, and is known to control alternative splicing of developmentally regulated genes. SNXA localizes to the nucleus but is excluded from the nucleolus in *A. nidulans*, paralleling the localization of hnRNP-M. The *snxA1* mutation was originally identified as an extragenic suppressor of mutations in the G2/M regulatory gene $nimX^{cdc2}$, and we have shown that it suppresses mutations in multiple components of the CDC2/CYCLINB regulatory pathway, including $nimT23^{cdc25}$ and $nimE6^{cyclinB}$ but not *nimA5* or *nimA1*. We further found that both *snxA1* and a second allele, *snxA2*, are hypomorphic and that both mutations result in significantly decreased *snxA* transcription and SNXA protein levels. Additionally, mutation or deletion of *snxA* alters $NIME^{CYCLINB}$ localization patterns. Our data suggest that SNXA may normally function to restrain the G2/M transition by affecting the CDC2/CYCLINB regulatory pathway, suggesting a novel function for the SR/RRM family that links RNA metabolism and transport to regulated cell division.

Teaching in a digital world: Developing an online course in Genetics. Indrani Bose. Biology, Western Carolina University, Cullowhee, NC.

Online courses have the advantage of being able to reach a wide audience. In addition, both traditional and non-traditional students are increasingly demanding courses where they can master the material at their own pace. However, research has shown that designing an engaging online course is often the roadblock to retention and good student learning outcomes. This talk will present some of the strategies being used to develop a Genetics course for non-majors. This will include ways to engage students, to assess them in a digital environment, and to determine whether quality controls are being followed. These strategies are being used by teachers in the University of North Carolina system to design online courses in Humanities as well as in the Sciences.

Educating beyond undergrads and graduate students: mycology meets social media. Lynne Boddy[1], Cat Adams[2]. 1) Sch Biosci, Cardiff Univ, Cardiff, United Kingdom; 2) University of California, Berkeley, USA.

As researchers it is now essential, and for some even compulsory, to communicate our research and knowledge of our subject to a wide audience. Though we may be effective communicators with other biologists and students, communicating our message to the general public requires different approaches, for which our abilities often fall short. Historically, scientists have engaged with the public through writing popular books, participating in radio and TV programs, speaking to groups like natural history clubs or classrooms, and creating semi-permanent exhibitions such as those in museums or botanical gardens. More recently, the advent of social media has produced novel ways to communicate to an extremely broad audience with very little time or effort. We will introduce some of these social media platforms, focusing on Twitter, blogs, and freelancing for popular science news sources. We will explain what each of these social media platforms is best used for, who is using them, why you should use them, the basics of how to use them, and give concrete tips for excelling at each of the platforms. Finally, we will show how social media can be used for crowd sourcing, including fundraising for research. Still not convinced? Data show that scientists who use social media on a regular basis get more grants.

Investing in the Fungal Genetics Stock Center: Kansas State Plant Pathology offers new home for collection. Kevin McCluskey, John Leslie. Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, KS.

In response to changes in federal support for living collections, the Fungal Genetics Stock Center relocated in November 2014 to the department of Plant Pathology at Kansas State University. The relocation included over thirteen thousand pounds of equipment and materials and was accomplished in one day. Despite this significant disruption, the collection continued to provide high quality resources with very few delays.

With over 25,000 accessioned fungal strains including over 13,000 gene deletion and molecularly modified (GMO) strains, the FGSC is the largest collection of genetically characterized and manipulated fungal strains in the world. In addition to these accessioned strains, the FGSC holds 775 plasmids and nearly 5,000 deletion mutants for Cryptococcus or Candida contributing to its global leadership in curating and distributing materials to researchers around the world. Existing collections at Kansas State complement the FGSC holdings and emphasize the synergy of bringing the FGSC to KSU.

Keeping Teaching Fresh: Resources for new ideas and approaches in the classroom. Marilee Ramesh. Department of Biology, Roanoke College, Salem, VA.

Good teaching is a constant work-in-progress. Effective instructors strive to update content, incorporate new methods of delivery, and respond to a changing student audience. As instructors, we try to keep our teaching fresh by trying new approaches in the classroom based on new pedagogy or ideas from colleagues. It can be a challenge to keep current with the best teaching practices for our field. Our colleagues can be a valuable source for new ideas and advice for what has worked and not worked for them. An overview of some of the resources members of our community have found useful to support instruction will be presented with attention to the topics of fungi and genetics, as well as scientific inquiry.

Friday, March 20 3:00 PM–6:00 PM
Merrill Hall

Mating Systems and Sexual Development
Co-chairs: Paul Dyer and Alex Idurm

Constraints on sex by a single mating-type: a case study from lichenized fungi. Ioana Onut Brännström[1], Sandra L. Ament[1], Toby Spribille[2], Douglas G. Scofield[1], Hanna Johannesson[1]. 1) Uppsala University, Uppsala, Sweden; 2) University of Graz, Graz, Austria.

An organism's reproductive behavior is expected to have a major impact on its ability to adapt and colonize new areas. Although the theoretical predictions on the topic are clear, empirical data are needed in order to link reproductive mode with ecology of different taxa. In this study we focus on the reproductive characteristics of lichenized fungi: symbiotic lineages that associate with algae and/or cyanobacteria to form an intimate biological union. Our study focuses on the family Icmadophilaceae. In this family, multiple transitions from sexuality to asexuality have taken place – as judged by the species phylogeny and presence or absence of fruiting structures. These transitions appear to be accompanied by a shift in morphology from crustose thalli to upraised white-colored thalli, possibly releasing them from substrate constraints. We sequenced the genomes and transcriptomes of three lichenized fungi and one lichen within Icmadophilaceae and used the architecture of their mating-type idiomorphs to gain insight into their mating systems. Both putative asexual species included in the study (*Thamnolia vermicularis* and *Siphula ceratites*) harbor mating-type idiomorphs consistent with a heterothallic mating system. *T. vermicularis* is a cosmopolitan species encountered in alpine or arctic environments where it often forms extensive colonies. When screening a sample of 218 individuals of *T. vermicularis* covering the whole Northern hemisphere and parts of Southern hemisphere, we find only one of the mating types (Mat 1-2), suggesting that sexual reproduction is constrained by the existence of a single mating type in this species. We hypothesize that this pattern is the result of the spreading of a well-adapted clone from a heterothallic and sexual ancestor, possibly preceded by a demographic bottleneck.

Mating-type genes in cereal rust fungi. Guus Bakkeren[1], John Fellers[2], Rob Linning[1], Les Szabo[3], Scot Hulbert[4], Xianming Chen[4,5], Brent McCallum[6], Xiben Wang[6], Richard Hamelin[7], Barry Saville[8], Christina Cuomo[9]. 1) Pacific Agri-Food Res Ctr, Agriculture & Agri-Food Canada, Summerland, BC, Canada; 2) USDA-ARS, Kansas State U., Manhattan, KS, USA; 3) USDA-ARS, Cereal Disease Laboratory, U. of Minnesota, St Paul, MN, USA; 4) Washington State U., Pullman, WA, USA; 5) USDA-ARS, Wheat Genet., Qual., Physiol. & Dis. Res. Unit, Pullman, WA, USA; 6) Agriculture & Agri-Food Canada, Morden, MB, Canada; 7) Forestry Dept., U. of British Columbia, BC & Natural Resources Canada - Québec, QC, Canada; 8) Trent U., Peterborough, ON, Canada; 9) Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Rust fungi have complex life cycles and many complete their sexual and asexual stages on different host plants. Because of their biotrophic life style, ephemeral, enigmatic sexual stage, and recalcitrance to molecular manipulation, nearly no data exists on their mating type systems: cereal rust fungi may display bipolar mating behavior. Using generated genome sequences of the wheat-infecting rusts *Puccinia graminis tritici* (stem rust), *P. striiformis tritici* (stripe rust) and several *P. triticina* (*Pt*, leaf rust) isolates, we shed light on structural features of their mating-type genes. Two divergently transcribed homeodomain (HD)-containing gene pairs are found in each dikaryotic isolate, similar to the paradigm established for *Ustilago* species. Functional characterization of these *Pt* genes shows that their expression can switch heterologous *Ustilago* cells to filamentous growth, indicative of productive mating interactions. Three related pheromone receptor (*Pra*) genes are found in each dikaryotic cell, supporting the hypothesis that tri-allelic recognition systems may be ancestral in basidiomycetes. In the fragmented genomes, no clear linkage between the HD and *Pra* genes is found; in 30 Canadian *Pt* field isolate genomes, 6 HD allele pairs are distinguished, indicating multiple mating types exist and possibly a *pseudo-bipolar* system

encompassing loose linkage as has been described for some Microbotryomycetes. Finally, RNAseq data comparing the sexual stages represented by aecio- and pycniospores produced on the alternate host to wheat infection reveal stage-specific transcripts, including effectors.

Chromosomal inversion-based mechanism of mating type switching in *Hansenula polymorpha*. Hiromi Maekawa, Yoshinobu Kaneko. Graduate School of Engineering, Osaka University, Suita, Osaka, Japan.

The mating system of Saccharomycotina has evolved from the ancestral heterothallic system as seen in *Yarrowia lipolytica* to homothallism as seen in *Saccharomyces cerevisiae*. The acquisition of silent cassettes was an important step towards homothallism. However, some Saccharomycotina species that diverged from the common ancestor before the acquisition of silent cassettes are also homothallic, including *Hansenula polymorpha*. We investigated the structure of the mating type locus (*MAT*) in *H. polymorpha* and found two *MAT* loci, *MAT1* and *MAT2* that are ~18 kb apart on the same chromosome. The chromosomal location of *MAT1* and *MAT2* was found to influence their transcriptional status, with only one locus maintained in an active state. A histone deacetylase homologous to *S. cerevisiae* Sir2 had no role in this transcriptional repression, suggesting a silencing mechanism distinct from that in *S. cerevisiae*. A chromosomal inversion of the *MAT* intervening region was induced under mating condition and resulted in the switching of the two *MAT* loci and hence of mating type identity, which was required for homothallism. This chromosomal inversion-based mechanism represents a novel form of mating type switching that requires two *MAT* loci, of which only one is expressed. Furthermore, we investigated the function of the mating type genes. *MAT1*-encoded α1 and *MAT2*-encoded **a**2 specifies α and **a** cell identity respectively, and are required for mating. *MAT1*-encoded α2 and *MAT2*-encoded **a**1 were essential for meiosis. α2 gene was expressed in haploid cells as well as diploid cells. However, splicing of an intron that contains a stop codon occurred only in diploid cells, which may restrict α2 function to meiosis-competent diploid cells.

Identification of novel genes regulating sexual development in *Aspergillus* species by functional analysis of transcripts differentially regulated by mating-type loci. Nadhira Salih[1], Adel Ashour[1], Ryuta Wada[2], Junichi Maruyama[2], Katsuhiko Kitamoto[2], Paul Dyer[1]. 1) School of Life Sciences , University of Nottingham, Nottingham NG7 2RD, United Kingdom; 2) Department of Biotechnology, The University of Tokyo, Tokyo 113-8657, Japan.

Sexual morphogenesis in filamentous ascomycete fungi requires the co-ordinated activity of developmental pathways encompassing mating, fruit body production, and meiosis and final generation of ascospores. It has been estimated that at least 400, and probably a much higher number, of genes are needed for normal sexual development. However, a much lower number of genes have so far been identified, indicating that many genes required for sexual morphogenesis remain to be characterised. It is of interest to gain information about such genes as they can provide both fundamental insights into sexual development, and might also provide practical clues as to the genetic basis of asexuality in fungi of applied importance as animal and plant pathogens, and as species used in the biotechnology industry. In the case of *Aspergillus* species approximately 80 genes have so far been described with a proven role in sexual development. Using idiomorph replacement and microarray-based approaches over 30 genes showing greater than 10-fold difference in expression between *MAT1-1* and *MAT1-2* mating types of *A. oryzae* were identified, most of these being of unknown function. We speculated that many of these genes might be required for sexual development. A systematic gene deletion study was therefore undertaken in which candidate genes were deleted from the homothallic (self-fertile) model species *A. nidulans*, and any effect on normal sexual development determined. This led to the discovery of at least 10 novel genes which were required for normal sexual development. Deletion of some genes resulted in total sterility whereas for others a modulation in levels of fertility was evident, suggesting different classes of activity. Gene function was confirmed by complementation by sexual crossing. A model is proposed as to how the newly identified genes relate to those previously involved in sexual development of *Aspergillus* species.

Biological Functions of Fungal Unisexual Reproduction: Sex Before Sexes. Joseph Heitman. Dept of Molecular Genetics and Microbiology (MGM), Duke University, Durham, NC. heitm001@duke.edu.

Sexual reproduction drives eukaryotic genetic exchange. Sex evolved early and was extant in the last eukaryotic common ancestor (LECA). Fundamental principles of sex are conserved (ploidy change, meiosis, mate recognition), yet some facets are ancestral, others derived. Many species have two mating-types or sexes. Others have more, including 3 in slime mold, 7 in *Tetrahymena*, and >1000 in some fungi. Diverse *MAT* loci, sex chromosomes, and sex determinants suggests sexual specification itself may not be ancestral. We study a new mode of sexual reproduction involving just one mating-type, unisexual reproduction, which occurs in pathogenic *Cryptococcus* species. These human fungal pathogens are basidiomycetes descended from a tetrapolar mating-type ancestor with 1000s of mating-types, yet this pathogenic clade has just two mating-types, **a** and alpha. Moreover the global population is largely just alpha mating-type. This posed a conundrum of how they might complete a sexual cycle; it was long thought they might be largely asexual. Instead we discovered they have an unusual unisexual cycle involving only one mating-type. Like opposite sex, unisex can admix parental diversity in progeny. However, in other cases solo unisex selfs identical genomes with no genetic diversity to exchange. Why organisms do so challenges conventional models. We find unisex can provide adaptive benefit. First, unisex generates genetic diversity de novo. Second, unisex promotes yeast-hyphae transition, enabling nutrient foraging. Third, unisex reverses Muller's Ratchet, avoiding deleterious mutation accumulation. Fourth, unisex overcomes Hill-Robertson effects, separating beneficial from deleterious mutations and linking beneficial mutations. Other fungi and parasites also reproduce unisexually. Unisex may have evolved to mitigate costs of sex and yield benefits of conventional sex. Studies of fungal sex illustrate general principles relevant to model and pathogenic microbes and multicellular eukaryotes, and suggest the LECA may have been unisexual. In this view, unisex may be both ancestral and recently rederived. If so, then there was an evolutionary epoch featuring sex before sexes.

Control of sexual and asexual development in *Aspergillus nidulans* by two different modules: MAPK (SteC-SteD-MkkB-MpkB) and heterotrimeric VapA-VipC-VapB methyltransferase complex. Oezguer Bayram. Department of Biology, Maynooth University, Maynooth, Kildare, Ireland.

Fungal development and secondary metabolite production are regulated by environmental cues such as solar light, CO_2/O_2 ratio, pH, carbon source, nitrogen source and starvation. Environmental messages are transduced by signaling complexes from plasma membrane to the nucleus where the regulatory downstream elements e.g. transcription factors are activated or inactivated in order to respond to the coming signal. Filamentous fungus *Aspergillus nidulans* produce asexual spores (conidia) in the presence of light whereas it reproduces sexually in the darkness generating fruiting bodies (cleistothecia). *A. nidulans* has evolved two different signaling pathways to control two developmental programs. First pathway uses phosphorylation to transmit the signals. Mitogen Activated Protein Kinase (MAPK) pheromone response module controls sexual development and secondary metabolism. The MAPK module, which consists of SteC-SteD-MkkB-MpkB, is tethered to plasma membrane and migrates from membrane to nucleus to deliver the MAPK protein MpkB into the nucleus. MpkB phosphorylates the velvet family protein VeA that coordinates sexual fruit body formation and secondary metabolite production. Second pathway uses methylation/demethylation reactions to control asexual sporulation and secondary metabolism. Heterotrimeric VapA-VipC-VapB complex is also tethered to plasma membrane by zinc finger VapA protein that releases the methyltransferase heterodimers VipC-VapB in response to environmental stimuli. VipC-VapB enters into the nucleus where they influence histone modifications and chromatin state within the nucleus, which in turn controls asexual conidiation and sexual development. Both complexes use the plasma membrane as an attachment point, and traverse the cytoplasmic barriers and reach the nucleus to control different developmental programs and secondary metabolite production.

Genomics and transcriptomics to study connections between fruiting body development and secondary metabolism. Daniel Schindler, Stefan Gesing, Florian Altegoer, Ines Teichert, Ulrich Kück, Minou Nowrousian. Dept. of General & Molecular Botany, Ruhr University Bochum, Bochum, Germany.

Filamentous ascomycetes develop four major types of fruiting bodies that share a common ancestor, and a set of common core genes most likely controls this process. One way to identify such genes is to search for conserved genes and expression patterns. In a genome and transcriptome mining approach, we are using microarray and RNA-seq data from the Sordariomycetes *Sordaria macrospora* and *Fusarium graminearum*, and the Pezizomycete *Pyronema confluens*, as well as mutants from *S. macrospora* and from the Neurospora knockout project to identify genes that might play conserved roles in fruiting body morphogenesis. Among the genes that we identified are genes involved in the regulation of chromatin-associated processes, for example the histone chaperone gene *asf1* and the transcription factor gene *pro44*, as well as secondary metabolite genes including several melanin biosynthesis genes and the polyketide synthase gene *pks4*. The melanin genes are required for the black pigmentation of fruiting bodies and ascospores, whereas *pks4* turned out to be essential for correct fruiting body morphology. Deletion of *pks4* leads to sterility in *N. crassa* as well as *S. macrospora*. The *S. macrospora* mutant is able to form protoperithecia (young fruiting bodies), but no mature fruiting bodies or spores. Surprisingly, overexpression of *pks4* in *S. macrospora* results in enlarged, malformed fruiting bodies. Thus, correct expression levels of *pks4* are essential for wild type-like perithecia formation. The predicted PKS4 protein has a domain structure that is similar to homologs in other fungi, but conserved residues of a methyl transferase domain present in other fungi are mutated in PKS4. Expression of several developmental genes is misregulated in the *S. macrospora pks4* mutant. However, the development-associated *app* gene is not downregulated in the mutant, in contrast to all other previously studied mutants with a block at the protoperithecial stage. One might speculate that the yet unknown metabolite produced by PKS4 plays a regulatory role in fruiting body development.

Complex formation of RNA silencing proteins in the perinuclear region of *Neurospora crassa*. Logan Decker[1], Erin Boone[1], Hua Xiao[1], Benjamin Shanker[1], Shannon Boone[1], Shanika Kingston[2], Seung Lee[1], Thomas Hammond[3], Patrick Shiu[1]. 1) Biological Sciences, University of Missouri, Columbia, MO; 2) Department of Biology, Barry University, Miami Shores, FL; 3) School of Biological Sciences, Illinois State University, Normal, IL.

The filamentous fungus *Neurospora crassa* is made up of interconnected cells where nuclei and other cellular components share a common cytoplasm. This trait, while beneficial for distributing resources, may promote the spread of detrimental elements such as transposons and viruses. Perhaps for this reason, *Neurospora* possesses several surveillance mechanisms that operate during different phases of its lifecycle. One of these defense mechanisms is known as meiotic silencing by unpaired DNA (MSUD). In MSUD, genes not paired during meiosis are targeted by a post-transcriptional gene silencing pathway. Here, our bimolecular fluorescence complementation (BiFC) study suggests that common RNAi proteins (RNA-directed RNA polymerase, Dicer, and Argonaute) as well as others form a meiotic silencing complex in the perinuclear region (the “surveillance checkpoint”), with intimate interactions among the majority of them. We have also shown that SAD-2 (a putative scaffold protein) is likely the anchor for this assembly.

Friday, March 20 3:00 PM–6:00 PM
Chapel

In vivo Imaging of Host-pathogen Interactions
Co-chairs: Matthias Brock and Barbara Valent

***In vivo* visualisation of fungal infections: An introduction to strain construction, imaging and challenges.** Matthias Brock. Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knoell Inst, Jena, Germany.

In recent years several advances have been made to visualise the infection process from fungal pathogens under *in vivo* conditions. *In vivo* imaging allows the investigation of time-resolved interactions of a pathogen with its host and provides temporal and spatial information on disease progression or successful clearance. Fluorescence and bioluminescence imaging make up the major proportions of currently used *in vivo* imaging techniques, but all systems have advantages and disadvantages that need to be analysed prior to selection of a specific system. While fluorescence imaging is the method of choice in intravital microscopy or the investigation of *in vivo* host pathogen interactions in transparent animals such as the zebrafish model, high background fluorescence significantly impacts its use in murine infection models. Therefore, investigation of disease progression and therapy monitoring in murine models mainly relies on the use of light-emitting luciferases. Although the spatial resolution from bioluminescence imaging is far below the resolution from fluorescence imaging, it is an extremely sensitive method due to very low background signals. To introduce the power of bioluminescence imaging, monitoring of invasive aspergillosis and disseminated candidiasis in temporal and spatial resolution will be shown. Additionally, suggestions will be given that may be suitable to increase the sensitivity of bioluminescence imaging systems and its potential use in studying disease caused by plant pathogenic fungi.

Bioluminescence imaging of *Candida albicans* infections. Christophe D'Enfert[1,2]. 1) Institut Pasteur, Fungal Biology and Pathogenicity, Mycology Department, Paris, France; 2) INRA, USC2019, Paris, France.

Bioluminescence imaging allows the visualization of the temporal and spatial progression of biological phenomena, in particular infection, by non-invasive methods *in vivo*. This nature-borrowed technology has been successfully used to monitor bacterial infections. It has now been extended to the tracking of fungal infections such as those caused by the two major opportunistic fungal pathogens *Candida albicans* and *Aspergillus fumigatus*. Notably, the *gLUC59* reporter gene was developed by fusing a synthetic, codon-optimized version of the *Gaussia princeps* luciferase gene to the *C. albicans PGA59* gene, which encodes a glycosylphosphatidylinositol-linked cell wall protein (1). This allows cell surface exposure of the luciferase and its detection in intact cells. The *gLUC59* reporter has now proven a convenient tool to study several aspects of *C. albicans* infection and biofilm formation in animal models. This will be illustrated and the pros and cons of the *gLUC59* reporter will be discussed.

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In vivo dynamics of Candida-immune interaction in the zebrafish. Robert Wheeler, Remi Gratacap, Joshua Jones, Sony Manandhar, Allison Scherer, Brittany Seman, Zachary Newman. Molecular & Biomedical Sciences, University of Maine, Orono, ME.

Current models of systemic candidiasis are limited in their application to questions of early host-pathogen interaction. We recently described a larval zebrafish model of candidiasis that provides a transparent and manipulable model for high-resolution non-invasive visualization of the innate immune-fungal interaction. Using this model we showed that interaction of *C. albicans* with phagocytes *in vivo* is different from that described *in vitro*, with macrophages able to control fungal growth only *in vivo*. We also demonstrated a novel role of NADPH oxidase in control of filamentous *C. albicans* growth. More recent experiments implicate NADPH oxidases in early immune recruitment to the *C. albicans* infection site, a previously unappreciated function of these important enzyme complexes.

We continue to utilize the larval zebrafish to approach other long-standing questions of fungal-host interaction. In one project we are modeling the more common but less dangerous types of candidiasis using an epithelial infection model that mimics key aspects of *in vitro* mammalian epithelial responses to *C. albicans*. Here, we have found differential immune responses depending on fungal burden by monitoring fungal infection concurrently with epithelial activation, inflammatory gene expression, and phagocyte recruitment.

Towards 4D-imaging of arbuscule development in rice. Ronelle Roth[1], Marco Chiapello[2], Jeremy Skepper[3], Uta Paszkowski[1,2]. 1) Plant Sciences, University Cambridge, Cambridge, United Kingdom; 2) Plant Biology, University Lausanne, Lausanne, Switzerland; 3) Cambridge Advanced Imaging Center (CAIC), Cambridge University, Cambridge, United Kingdom.

The oldest documentation of intracellular colonization of eukaryote cells by eukaryote invaders refers to 450 million years old fossil records of early land plants containing intracellular fungal feeding structures, so called arbuscules, within their cells. Arbuscules are the central structure of the mutually beneficial arbuscular mycorrhizal symbiosis that is occurring in most contemporary plants species. Mutualism is manifested in the bi-directional exchange of nutrients across the peri-arbuscular membrane. A symbiotic interface is established, composed of fungal and plant membranes and the intercalary matrix, that governs the inter-organismic molecular dialogue, controlling the trade of nutrients.

To investigate the membrane surfaces intimately involved in the fungus-induced reprogramming of the plant cell we apply in planta time-lapse live-cell imaging using Multi-Photon Confocal Microscopy (MPCM). To date, time-lapse live-cell imaging using standard confocal laser scanning microscopy in inner cortical root cell layers has been hampered by low resolution and photo-bleaching that impairs cell viability. In contrast, MPCM permits deep-tissue imaging at high resolution with minimal tissue damage. The data sets generated will provide a high resolution quantitative 4D reconstruction of membrane surfaces intimately involved in the intracellular plant-fungal dialogue.

Zoom into Nano: Super-Resolution Microscopy in Visualizing the 3D Architecture of Cell Walls and related Protein Complexes. Björn Sode[1], Dennis Eggert[2,3], Annemarie Glöckner[1], Marcel Naumann[1], Rudolph Reimer[2], Christian Voigt[1]. 1) Phytopathology and Biochemistry, Biocenter Klein Flottbek, Hamburg, Germany; 2) Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany; 3) Max Planck Institute for the Structure and Dynamics of Matter, Hamburg, Germany.

Polymeric cell walls have a fundamental function in the development und evolution of plants and fungi. They form the outside barrier to the environment and maintain integrity of cells, tissues and whole organisms. The architecture of cell walls is determined by the three-dimensional (3D) orientation of various polymers. New approaches in super-resolution fluorescence microscopy allow 3D, nanoscale visualization of distinct cell wall polymers. This information is used to reconstruct the polymeric network of cell walls based on exact experimental data. We present recent approaches in applying super-resolution microscopy on plant infection structures after fungal attack and on the cell wall of the plant pathogenic fungus *Fusarium graminearum* that revealed a previously unknown interaction and network formation of different types of glucan cell wall polymers; callose and cellulose in plants, chitin and callose in fungi. Combining these data with live cell imaging of glucan synthase complexes and their movement in the plasma membrane at nanoscale resolution, new insights into the regulation of cell wall biosynthesis is gained.

Subcellular reorganization during trichothecene mycotoxin induction in *Fusarium graminearum*. Marike Boenisch, Karen Broz, H.Corby Kistler. USDA ARS Cereal Disease Laboratory and University of Minnesota, 1551 Lindig Street, St. Paul, MN 55108, USA.

The ascomycete fungus *Fusarium graminearum* causes disease on wheat and barley and contaminates grain with trichothecene mycotoxins making it unfit for human consumption. Little is known about cellular and subcellular changes that occur during toxigenesis that may facilitate trichothecene synthesis and export. Recently, we demonstrated that three enzymes catalyzing early and late steps in trichothecene biosynthesis, hydroxymethylglutaryl CoA reductase (Hmr1p), trichodiene oxygenase (Tri4p), and calonectrin oxygenase (Tri1p), localize to spherical subcellular structures called "toxisomes" when grown in toxin inducing medium. The current study revealed that toxisomes also can be observed *in planta* during infection of wheat husks. Inoculation of paleas and glumes with conidia of a Tri4p::RFP strain reveal toxisomes in infection cushions and runner hyphae. *In vitro*, toxisomes co-localize with the endoplasmic reticulum (ER) as determined by using the fluorescent dye ER-Tracker Blue-White DPX. ER organization shifts from being highly reticulate under toxin non-inducing conditions to being tubular and with pronounced perinuclear ER upon toxin induction. Both ER-Tracker and Hmr1p::GFP fluorescence show similar reorganization during toxin induction. The spherical toxisomes surround nuclei as determined using a Tri4p::RFP/ histone H4p::GFP tagged strain. Thus, toxisomes appear to be perinuclear ER remodeled under toxin inducing conditions. For all three tagged enzymes examined, fluorescent patches co-localizing with ER-Tracker but not associated to nuclei are also visible under toxin induction. As a consequence, trichothecenes may be produced at both perinuclear and peripheral ER. In contrast to the biosynthetic enzymes, the export of trichothecenes is linked to endosomes. The trichothecene transporter Tri12p tagged with GFP localizes to motile vesicles, vacuoles and the plasma membrane based on co-localization with the fluorescent dyes CMAC and FM4-64. The biosynthesis and transport of trichothecenes in cellular compartments may play a role in sequestration of trichothecene molecules within the cell and self-protection from the toxin.

Infection structure-specific expression of lipase-like effector supports appressorial functionality and fungal cell-to-cell colonization of the rice blast fungus, *Magnaporthe oryzae*. E Oliveira-Garcia, B Valent. Plant Pathology, KSU, Manhattan, KS.

Rice blast caused by *Magnaporthe oryzae*, a hemibiotroph and facultative pathogen, is the most important disease of rice worldwide. At the early stage of infection, the germ-tube differentiates to form an appressorium immediately adjacent to the conidium. The appressorium serves for the direct penetration of the host. After host penetration, *M. oryzae* establishes a biotrophic interaction. It is assumed that different strategies employed by the fungus to avoid triggering defense responses, including masking of invading hyphae or active suppression of host defense mechanisms, are essential for a biotrophic parasitic lifestyle. During the infection process, *M. oryzae* secretes various effectors, which are hypothesized to be involved in effective host infection. To date, little is known about the influence of lipases during infection of plants by fungi. Here, we show that a lipase-like effector is up-regulated during plant penetration and biotrophic development. Using fluorescent protein tagging, we found lipases localized to stage-specific compartments at the host-pathogen interface. Importantly, we show that this lipase is focally secreted from the appressorial penetration pore into the O-ring before host invasion, revealing new levels of functional complexity for this fungal organ. Furthermore, we demonstrate that lipase-like effector accumulates massively in plant cell wall crossing points during cell-to-cell colonization by the fungus. This accumulation of the lipase-like effector at crossing points was observed during invasion of four consecutive rice cells following initial successful colonization of the first cell by the fungus. Based on these results we conclude that infection structure-specific expression of lipase-like effector supports appressorial functionality and/ or fungal cell-to-cell colonization. Our results also suggest a potential role of lipases for manipulation host cell channels, plasmodesmata, by the fungus.

Ralstonia solanacearum **lipopeptide induces chlamydospore formation followed by bacterial entry in close encounters with fungi.** Joe Spraker[1], Laura Sanchez[2], Pieter Dorrestien[2], Nancy Keller[3]. 1) Plant Pathology, University of Wisconsin - Madison, WI; 2) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California- San Diego, CA; 3) Bacteriology, Medical Microbiology and Immunology, University of Wisconsin- Madison, WI.

The polymicrobial consortium within the rhizosphere communicates by chemical signaling that, ultimately, impacts survival in symbioses. Here we characterize the endosymbiotic interaction of two economically important plant pathogens, *Aspergillus flavus* and *Ralstonia solanacearum*. Using a variety of histological techniques we show that fungal chlamydospore-like structures form in response to a diffusible compound produced by *R. solanacearum.* Imaging Mass-Spec (IMS) and targeted genetic deletion show this metabolite to be a new lipopetide named ralsolamycin. Confocal scanning laser microscopy with a GFP *R. solanacearum* isolate show bacterial internal colonization of chylamydospores, indicating a newly described endofungal lifestyle for this important plant pathogen.

Friday, March 20 3:00 PM–6:00 PM
Fred Farr Forum

RNA Metabolism and Transport
Co-chairs: Ane Sesma and Michael Bölker

mRNA transport meets membrane trafficking. Michael Feldbrugge. Institute for Microbiology, Heinrich-Heine University, Düsseldorf, NRW, Germany.

Active transport and local translation of mRNAs ensure the appropriate spatial organization of proteins within cells. Recent work has shown that this process is intricately connected to membrane trafficking. Here, we present new findings obtained in the model organism *Ustilago maydis*. In highly polarized cells of this fungus microtubule-dependent co-transport of mRNAs and endosomes is essential for efficient polar growth. We discuss a novel concept of endosome-coupled translation that loads shuttling endosomes with septin cargo, a process important for correct septin filamentation. Interestingly, evidence is accumulating that RNA and membrane trafficking are also tightly interwoven in higher eukaryotes suggesting that this phenomenon is a common theme and not an exception restricted to fungi.

Comparative transcriptomics of the human pathogen *Histoplasma* reveals conserved and widespread re-programming of transcript length. Sarah Gilmore, Mark Voorhies, Anita Sil. Department of Microbiology & Immunology, University of California, San Francisco, San Francisco, CA.

Eukaryotic cells integrate many layers of gene regulation beyond the initial transcription of a gene to coordinate complex cellular processes such as embryogenesis and cellular development; however mechanism of post-transcriptional gene regulation lag behind our understanding of transcriptional regulatory events. The thermally dimorphic human fungal pathogen *Histoplasma capsulatum* (*Hc*) exhibits readily reversible unicellular (budding yeast) and multicellular (hyphae) developmental states that are controlled by the environmental cue of temperature. Thus *Hc* represents an ideal organism to probe fundamental questions regarding the basic mechanisms of gene regulation that eukaryotic cells employ to cue multicellular development. In this work, we use the developmentally distinct cells types of *Hc* to uncover mechanisms of post-transcriptional gene regulation during development. Employing recent advances in RNA sequencing, de novo transcriptome reconstruction methodologies, and ribosome profiling (which measures translational efficiency genome-wide), we uncovered a novel means of post-transcriptional gene regulation in the yeast and hyphal cell types of *Hc*. Remarkably, we find that ~2% percent of the *Hc* genome exhibits differential 5' transcript ends (or leader sequences) between the two morphogenetic states. Comparative transcriptomics analyses of RNA sequencing data across multiple *Hc* lineages indicates that the majority of differential leader transcript architecture is conserved, suggesting that 5' transcript extensions are a non-random, biologically regulated process. Ribosome and mRNA density measurements uncovered a class of these longer leader transcripts that exhibit tight transcriptional and translational regulation. Further examination of this group of transcriptionally and translationally regulated genes reveals that some are involved in controlling *Hc* morphology and that their strict regulation may be necessary for the organism to make appropriate developmental decisions.

Regulating the regulators: Cleavage Factor I proteins in *Magnaporthe oryzae*. Julio Rodríguez-Romero, Ane Sesma. Centre for Plant Biotechnology and Genomics, Technical University of Madrid, Pozuelo de Alarcon, Madrid, Spain.

Cleavage factor I (CFI) proteins are core components of the polyadenylation machinery that can regulate several steps of mRNA life cycle, including alternative polyadenylation, splicing, export and decay. Rbp35 is a novel protein component of the polyadenylation machinery, and it is present exclusively in filamentous fungi[1]. In *Magnaporthe oryzae*, it regulates alternative polyadenylation of transcripts associated with pathogenicity. Here, we describe the regulatory mechanisms that control the Rbp35/CfI25 complex and Hrp1, another CFI protein. Using mutational, genetic and biochemical studies we demonstrate that cellular concentration of CFI mRNAs is a limited indicator of their protein abundance. Our results suggest that several posttranscriptional mechanisms regulate Rbp35/CfI25 complex and Hrp1 in the rice blast fungus, some of which are also conserved in other ascomycetes[2]. With respect to Rbp35, these include C-terminal processing, RGG-dependent localisation and cleavage, and a C-terminal autoregulatory domain. Our proteomic analyses indicate that Rbp35 also controls cellular levels of protein subsets but it is not required for general splicing or translation.Carbon depletion induces the transcription of two polyadenylated transcripts of ~1,000 (uORF1) and ~750 (uORF2) nucleotides in length that derive from *RBP35* 5'UTR. The uORF1 is required for correct function of the TOR kinase pathway on minimal media. The role of two additional CFI proteins in *M. oryzae*, Hrp1 and CfI25, is further analysed to understand why filamentous fungi have maintained proteins with apparently redundant functions. Our findings uncover broad and multilayer regulatory mechanisms controlling fungal polyadenylation factors, which have profound implications in pre-mRNA maturation.

References
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Switching the fate of mRNA transcripts for mitochondrial biogenesis in *Saccharomyces cerevisiae*. Chien-Der Lee, Benjamin Tu. UTSW, Dallas, TX.

PUF proteins are conserved post-transcriptional regulators that bind in a sequence-specific manner to the 3'UTRs of mRNA transcripts. Paradoxically, PUF proteins have been proposed to promote both degradation of their target mRNAs as well as their translation. Herein, we show how a yeast PUF protein Puf3p responds to glucose availability to switch the fate of its bound transcripts that encode proteins required for mitochondrial biogenesis. Upon glucose depletion, Puf3p becomes phosphorylated, associates with polysomes, and promotes translation of its target mRNAs. As such, nutrient-responsive phosphorylation toggles the activity of Puf3p to promote either degradation or translation of these mRNAs according to the needs of the cell. Such activation of translation of pre-existing mRNAs might enable rapid adjustment to environmental changes without the need for de novo transcription. Strikingly, a Puf3p mutant that prevents its phosphorylation no longer promotes mRNA translation but also becomes trapped in intracellular foci in an mRNA-dependent manner. Our findings suggest how the inability to properly resolve Puf3p-containing mRNA-protein granules via a phosphorylation-based mechanism might be toxic to a cell.

Mechanism of Quelling: Small Interfering RNA Production from Repetitive DNA. Qiuying Yang, Yi Liu. Dept Physiology, Univ Texas SW Med Ctr, Dallas, TX.

RNA interference is a conserved genome defense mechanism in eukaryotes that protects against deleterious effects of transposons and viral invasion. Repetitive DNA loci are a major source for the production of eukaryotic small RNAs but how these small RNAs are produced is not clear. Quelling in *Neurospora* is one of the first known RNAi-related phenomena and is triggered by the presence of multiple copies of transgenes. We show that DNA tandem repeats and double-stranded breaks are necessary and, when both are present, sufficient for triggering gene silencing and siRNA production. Introduction of a site-specific double-stranded break or DNA fragile site results in homologous recombination of repetitive sequences, which is required for gene silencing. In addition to siRNA production, the quelling pathway also maintains tandem repeats by regulating homologous recombination. Our study identified the mechanistic trigger for siRNA production from repetitive DNA and established a role for siRNA in maintaining genome stability.

Investigating the link between mRNA degradation and translation. Mark Caddick. Dept Biological Sci, Univ Liverpool, Liverpool, United Kingdom.

Within eukaryotes there are various mechanisms that silence transcripts. Generally these mechanisms promote both translational repression and rapid transcript degradation. For the majority of transcripts the key signal which triggers degradation and translational repression is shortening of the poly(A) tail to a critical length of about fifteen A nucleotides. This promotes coordinated translational repression and deadenylation dependent transcript degradation. Although translation promotes gradual deadenylation the rate of deadenlyation represents a major regulatory target, varying significantly between transcripts and different growth conditions. There are a number of examples where mRNA degradation is independent of deadenlyation including cell cycle regulated degradation of the histone mRNAs and nonsense mediate decay (NMD). NMD is a major quality control mechanism which triggers degradation of transcripts that contain a premature termination codon. In *A. nidulans* these three apparently distinct mechanisms, deadenylation dependent transcript degradation, NMD and cell cycle regulated degradation of histone mRNA, all induce the addition of non-templated pyrimidine residues at the mRNA 3' end (mRNA tagging). The addition of a 3' pyrimidine tag has been observed in plants animals and fungi and is likely to act by recruiting the Lsm-Pat1 complex to the transcript, which then initiates a cascade of events including translational repression, dissociation of the termination complex, decapping and both 5' and 3' mRNA degradation. Intriguingly in all cases tagging appears to be in part regulated by NMD components. Utilising molecular genetic techniques we are investigating different RNA degradation processes, assessing the role of various degradation pathways and addressing the question as to why NMD components should be involved. Our working hypothesis is that these NMD components provide a link with translational termination, linking mRNA degradation to translation.

Programmed stop codon readthrough leads to dually targeted protein isoforms. Alina C. Stiebler[1], Johannes Freitag[1,3], Kay O. Schink[2], Thorsten Stehlik[1,4], Julia Ast[1], Britta A. M. Tillmann[1], Michael Bölker[1,4]. 1) Biology, Philipps University, Marburg, Germany; 2) Faculty of Medicine, Centre for Cancer Biomedicine, University of Oslo, Montebello, Oslo, Norway; 3) LOEWE Excellence Cluster for Integrative Fungal Research (IPF), Senckenberg Society, Frankfurt am Main, Germany; 4) LOEWE Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany.

Translation of mRNA into protein is generally a very accurate process. However, programmed translational recoding is widely used in viruses to expand the coding capacity of their genomes. We have recently shown that in a wide range of fungal species programmed translational readthrough of stop codons is used to generate C-terminally extended isoforms of glycolytic enzymes. The extended isoforms carry a C-terminal signal sequence (PTS1) that mediates targeting to peroxisomes.

We characterized the sequence requirements for efficient translational readthrough and identified a short conserved stop codon context (UGA CUA). Genomic screening revealed that this motif occurs in a number of genes that encode important metabolic enzymes both in fungi and animals. We could show that ribosomal readthrough of these genes also results in the formation of peroxisomal isoforms. Overall, our data indicates that programmed stop codon readthrough is a common mechanism to reach a dual localization of enzymatic activity both in the cytosol and in peroxisomes in particular of enzymes implicated in redox homeostasis.

Light effects on the production and processing of RNA. Steven Woods, Yamini Arthanari, Christian Heintzen, Ray O'Keefe, Sam Griffiths-Jones, Sue Crosthwaite. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Light regulates the expression of a large number of genes in Neurospora. The light-signalling WHITE-COLLAR complex (WCC) induces a hierarchical network of transcription factors that propagate early and late light-induced changes in gene expression. Alternative splicing plays a key role in gene regulation and increasing protein diversity. Previous studies have demonstrated that alternative splicing of Neurospora transcripts occurs in response to changes in temperature, metabolites and pH but the possibility of light-induced alternative splicing has not yet been addressed. We have now investigated whether protein diversity is increased due to light-induced alternative splicing. We used RNA-Seq to profile the transcriptome of *Neurospora* across different regimes of light and dark. We see a range of transcriptional changes, including alternative splicing, intron retention, and alternative transcription start and end points.

Friday, March 20 3:00 PM–6:00 PM
Kiln

Stress Responses and Senescence
Co-chairs: Alfredo Herrera-Estrella and Heinz Osiewacz

Intervening into molecular quality control pathways: effect on fungal senescence. Heinz Osiewacz. Molecular Biosciences, J W Goethe University, Frankfurt, Germany.

Podospora anserina is a filamentous ascomycete with a limited lifespan. Lifespan is controlled by genetic, environmental and stochastic traits. Mitochondria play a key role in the processes leading to senescence and programmed cell death (PCD). During senescence, mitochondria accumulate functional impairments and change their morphology and ultrastructure. In the past, various pathways have been identified which are active in mitochondrial quality control. Strengthening these pathways by genetic interventions can improve mitochondrial quality over time and result in a delay of the induction of PCD and an increased lifespan. Sometimes, however, counterintuitive results are obtained indicating that more complex responses are induced by defined and specific interventions. Among the different pathways studied so far, those involved in the generation of reactive oxygen species (ROS) (1), the degradation of damaged proteins (2,3,4), the control of mitochondrial dynamics (5), autophagy (6) and the execution of PCD (7,8,9) have been found to be affective. In the lecture, I will summarize recent findings about the age-dependent mitochondrial membrane remodeling and changes in mitochondrial ultrastructure. In addition, work will be presented and discussed demonstrating the role of quality control in senescence and lifespan control. References (1) Kunstmann B, Osiewacz HD (2008) Aging Cell 7:651; (2) Luce K, Osiewacz HD (2009 Nat Cell Biol 11:852; (3) Weil A et al. (2011) Cell Cycle 10:4280; (4) Fischer F et al. (2013) Nat Commun 4:1397; (5) Scheckhuber C et al. (2007) Nat Cell Biol 9: 99; (6) Knuppertz L et al. (2014) Autophagy 10: 822; (7) Hamann A et al. (2007) Mol Microbiol 65: 948; (8) Brust D et al. (2010) Aging Cell 9: 761; (9)Daum B et al. (2013) PNAS 110: 15301

Mitochondria-mediated stress tolerance and senescence in *Botrytis*. Rachl Tetroashvili, Liat Oren-Young, Amir Sharon. Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel.

Mitochondria seems to play a major role in determination of cell fate in lower eukaryotic organisms, and specifically in fungi. Alterations in mitochondria homeostasis and metabolic activities occur in response to intracellular cellular changes and affect a range of cellular processes, including senescence and PCD. Studies in *Saccharomyces cerevisiae* showed that mutation that impair mitochondria homeostasis resulted in enhanced stress sensitivity, senescence and PCD. The end result, namely early senescence and PCD or survival of the cells, is mediated by anti-PCD machineries, but it is unclear how the two systems are coordinated. In *S. cerevisiae* and *Botrytis cinerea*, the most significant anti-apoptotic proteins are Bir1p and BcBir1, respectively.

To try and connect between the mitochondria-driven PCD-promoting signals and the anti-apoptotic machinery, we generated *B. cinerea* mutants in the genes *BcCYC* and *BcLON1*, homologues of *S. cerevisiae UME3*, a non-essential cyclin that affects mitochondria fission and *LON1*, a mitochondria-residing serine protease that degrades mitochondria proteins. Mutants were characterized and showed alterations in responses to stress and enhanced senescence. Next we produced double mutants, which over express the BcBir1 protein. If BcBir1 mediates the mitochondria-produced signal, we expect that the hyper sensitivity to stress and enhanced senescence of the single mutants will be at least partially reversed. The results of this study will be presented and discussed.

Induced programmed cell death in *Neurospora crassa*. Arnaldo Videira[1,2]. 1) ICBAS-Instituto de Ciências Biomédicas de Abel Salazar, University of Porto; 2) IBMC-Instituto de Biologia Molecular e Celular, University of Porto.

Treatment of *Neurospora crassa* with drugs like phytosphingosine or staurosporine can induce programmed cell death (PCD), which typically includes reduced viability, ROS production, glutathione efflux and DNA condensation and fragmentation. Transcriptional profiling of drug-treated fungal cells revealed proteins associated with PCD development. This knowledge can be used to modulate the PCD process in *N. crassa*, other fungi and human cells. Exposing cells to a combination of death inducers with drugs targeting specific proteins involved in the death process leads to the enhancement of PCD, thus having anti-fungal and anti-tumor potential. A major effect of phytosphingosine is a repression of genes encoding mitochondrial proteins. Staurosporine highly induces the expression of a gene encoding a transporter of the ABC type, responsible for drug extrusion from the cells. Its absence renders cells hypersensitive to the drug. Another gene up-regulated by staurosporine is a novel transcription factor that appears to regulate drug resistance and cell death in laboratory strains and in wild isolates of *N. crassa*. Staurosporine also provokes alterations in intracellular calcium in a process mediated by the

phospholipase C signaling pathway that includes calcium influx and its mobilization from and to internal stores and leads to a defined cytosolic Ca^{2+}-signature. In agreement, staurosporine sensitivity is altered in deletion mutants lacking proteins implicated in calcium handling. Respiratory chain mutants (like strains lacking subunits of complex I or a Ca^{2+}-dependent alternative NAD(P)H dehydrogenase) are hypersensitive to staurosporine and display a deficient cytosolic Ca^{2+}-signature. These results highlight the importance of the involvement of mitochondria and bioenergetics in the PCD process.

Golgi-localized and the palmitoyl transferase-related AkrA homologs mediates $[Ca^{2+}]_i$ transient to response ER and azole stresses. Yuanwei Zhang[1], Qingqing Zhen[1], Jinxing Song[1], Lina Gao[1], Alberto Muñoz[2], Nick D. Read[2], Ling Lu[1]. 1) College of Life Science, Nanjing Normal Univeristy, Nanjing, jiangsu, China; 2) Manchester Fungal Infection Group, Institute of Inflammation and Repair, CTF Building, University of Manchester, Manchester M13 9NT, UK.

Finely tuned $[Ca^{2+}]_i$ changes mediate several intracellular functions, resulting in subsequent activation or inactivation of a series of conserved Ca^{2+} signaling components and their target proteins. Palmitoylation is a reversible post-translational modification involved in membrane protein trafficking and functional modulation. However, studies on the relationship between calcium signaling and palmitoylation have been limited. Here, we demonstrate that the homologs of yeast palmitoyl transferase ScAkr1p, AkrA in *Aspergillus nidulans* and SidR in *Aspergillus fumigatus*, play important roles under low calcium conditions. Deletion of *akrA* or *sidR* shows remarkable defects in hyphal growth and conidiation, but adding extracellular calcium can completely rescue the growth defects. Moreover, using the calcium probe aequorin in live cells, we found that all of the palmitoyl transferase-related *akrA* mutants induced larger decreases in the $[Ca^{2+}]_i$ response to extracellular Ca^{2+} compared to the previously identified high-affinity calcium influx system members (CchA and MidA) and compared to the parent control strain. Moreover, ER stressors- or azole-induced calcium transient was completely blocked by AkrA defects, especially in low calcium conditions where we did not detect a calcium transient. Interestingly, all of the above-described functions AkrA are tightly related to cysteine residues in its DHHC-CRD and its palmitoyl transferase activity. Thus, Golgi-localized AkrA mediates the $[Ca^{2+}]_i$ transient likely by globally palmitoylating calcium signaling components and their target proteins. Our findings provide insight into a new link between calcium signaling and palmitoylation in the regulation of cell survival processes upon ER and membrane stress.

Proteome analysis of transiently oxidized proteins during the hyperoxidant state that triggers conidiation in *Neurospora crassa*. Wilhelm Hansberg[1], Teresa Nava-Ramírez[1], Bastian Jöhnk[2], Oliver Valerius[2], Gehard Braus[2]. 1) Instituto de Fisiologia Celular, Universidad Nacional Autónoma de Mexico, UNAM, Mexico City, Mexico; 2) Institute for Microbiology and Genetics, Georg August University of Göttingen, Göttingen, Germany.

The *Neurospora crassa* asexual cell cycle is stated by filtering an exponentially growing liquid culture and exposing the resulting mycelial mat to the air. The increase in oxygen tension causes oxidative stress in the air-exposed hyphae. Total protein is oxidized during the first 0-10 minutes air exposure followed by protein degradation and resynthesis. Oxidative stress results in hyphal adhesion during the first 40 minutes of air exposure. Using a method to identify proteins with reversible oxidized cysteine residues, we have isolated the cysteine-derivatized proteins at different times of the conidiation process (0, 2, 5, 10, 30 and 60 min air exposure) and identified them by mass spectrometry. A positive control, derivatized proteins from a culture treated 10 min with 20 mM H_2O_2, and a negative control, without derivatizing agent, were included. About 500 proteins were identified that increased in relative amount at 2 - 10 min air exposure. A functional analysis of these proteins will be presented. Results revealed a rapid, transient and extensive response to oxidative stress. Proteins detected are consistent with: growth arrest, unfolded protein response, protein degradation at the ER, vacuole and the proteasome, important mitochondrial and carbon metabolism regulation.

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Stress signaling in *Botrytis cinerea:* The response regulator BcSkn7. Anne Viefhues, Ines Schlathoelter, Paul Tudzynski. IBBP, University of Muenster, Muenster, Germany.

In the course of plant infection pathogens trigger an oxidative burst, which is part of the plants early defense reaction. The necrotrophic plant pathogen *Botrytis cinerea* is known to contribute actively to the release of reactive oxygen species (ROS). ROS have an ambivalent role as they are on the one hand toxic and responsible for damages of biological molecules and on the other hand they are important second messengers. For the integration and transmission of these signals several stress responsive components are necessary to evoke the appropriate response.

Two key players in the oxidative stress response are the transcription factor Bap1[1] and the response regulator BcSkn7. Phenotypic analysis of *bcskn7* and *bap1bcskn7* deletion mutants showed alterations in differentiation, virulence and gene expression. Δbcskn7 is reduced in vegetative growth and affected in the formation of reproduction structures. The mutant is highly sensitive to oxidative stress, as well as reacts to temperature and osmotic changes. Modifications in the composition of the cell wall could be detected, indicated by changed gene expression, reduced protoplast formation and sensitivity to cell wall or membrane stressors. Furthermore, an enhanced secretion of ROS could be noticed. Virulence of the mutants based on conidia is not affected, however mycelium derived infections are defective. This effect is even more severe for ΔΔbap1bcskn7 and is probably due to a reduced penetration ability of the infection cushions. Expression analyses revealed a strong influence of Bap1 and BcSkn7 on the regulation of oxidative stress responsive genes. In a Y1H approach a direct binding to the promotors of *gsh1* and *grx1* by Bap1 and of *glr1* by BcSkn7 could be verified. Next steps include the examination of a direct interaction between Bap1 and BcSkn7 as they seem to act in concert in gene regulation.

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Mechanical stress initiates intercalary growth in *Epichloë* fungal symbionts of grasses. K. G. Sameera 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) Plant Fungal Interactions, AgResearch Grasslands, Palmerston North, Manawatu, New Zealand; 2) Institute of Fundamental Studies, Massey University, Palmerston North, New Zealand; 3) School of Medical Sciences, University of Aberdeen, United Kingdom; 4) Manchester Fungal Infection Group, University of Manchester, United Kingdom; 5) AgResearch, Lincoln Research Centre, Christchurch, New Zealand.

Colonization of aerial grass tissues by seed-transmitted *Epichloë* endo-symbionts initially occurs through ramification of hyphal tips between cells of the host shoot apical meristem (SAM). Uniquely, when hyphae in the SAM start to invade developing leaves, growth ceases at apices, and hyphae extend via intercalary growth (division and extension in non-apical compartments). We hypothesise that intercalary growth is stimulated by mechanical stretch imposed on hyphae by their attachment to elongating host cells, and that this stress is sensed by mechano-sensors located on hyphal membranes. Deletion of *E. festucae mid1*, a putative orthologue of the *mid1* yeast mechano-sensor, and a component of the Mid1/Cch1 calcium channel, reduced *E. festucae* radial growth rate in culture, caused aberrations in hyphal cell walls, and greatly restricted intercalary growth in infected plants. A technique to mimic the hyphal stretching proposed to occur *in planta* has been developed and tested on wild type *E. festucae* growing in culture. Intercalary compartments remained viable despite being stretched to 20% of their original length, and stretching also initiated *de novo* mitosis and septation in intercalary compartments. Calcium imaging experiments on *E. festucae* growing in culture have revealed that the Mid1 protein is responsible for calcium pulses at the hyphal tip during growth, and that the calcium originates from the exterior of the hypha and not from calcium stores. Studies are underway to characterise calcium signalling in intercalary compartments in wild type and the Δ*mid1* deletion mutant when subjected to mechanical stress.

Damage-associated molecular patterns and small RNAs in the response to injury of *Trichoderma atroviride*. E. Medina-Castellanos[1], J. Villalobos-Escobedo[1], M. Heil[2], C. Abreu[1], A. Herrera-Estrella[1]. 1) National Laboratory of Genomics for Biodiversity, CINVESTAV-IPN, Irapuato, Mexico; 2) Irapuato Unit, CINVESTAV-IPN, Irapuato, Mexico.

The response to damage is crucial for the survival of multicellular organisms, enabling their adaptation to hostile environments. *Trichoderma atroviride*, responds to mechanical damage by activating regenerative processes and conidiation. During this response, reactive oxygen species (ROS) are produced by a NADPH oxidase complex (Nox1/NoxR). To understand the molecular mechanisms underlying this process, we evaluated molecules such as extracellular ATP (eATP) and Ca^{2+} that could trigger wound-induced conidiation and investigated the activation of mitogen-activated protein kinase (MAPK) pathways induced by eATP, Ca^{2+} and ROS. Since eATP triggers wound-induced conidiation, we propose that it representa a damage-associated molecular pattern (DAMP), which is released from damaged hyphae. We have shown that eATP promotes Nox1-dependent production of ROS, activates a MAPK pathway and induces conidiation. Mutants in the MAPK-encoding genes *tmk1* and *tmk3* are affected in wound-induced conidiation. Phosphorylation of both Tmk1 and Tmk3 is triggered by eATP, whereas Ca^{2+} signaling appears to participate downstream in an independent pathway. Our data support the existence of a potential ATP-specific receptor to sense eATP. eATP and Ca^{2+} activate different pathways that converge to regulate conidiation genes. Thus, the early steps of the damage response in *T. atroviride* share conserved elements with those known from plants and animals. Given the observed transcriptional response to injury and the existence of the highly conserved mechanism of regulation of gene expression based on RNAi in *Trichoderma*, we decided to analyze the role of the components of the RNAi machinery and small RNAs in this process. Analysis of gene replacement mutants in all components of the machinery revealed that Dcr2 and Rdr3 play a major role in injury induced conidiation. Comparative analyses of the small RNAs produced during injury in the wild type and Δ*dcr*2 strains in response to injury revealed major differences, suggesting the involvement of microRNAs in this process.

Friday, March 20 3:00 PM–6:00 PM
Heather

Early Diverging Fungi
Co-chairs: Tim James and Naomi Fast

Ancient pectinases equipped ancestral fungi to digest plant cell walls. Mary Berbee[1], Ying Chang[1], Sishuo Wang[1], Satoshi Sekimoto[1,2], Joseph Spatafora[3]. 1) Dept Botany, Univ British Columbia, Vancouver, B.C., Canada; 2) Biological Resource Center, NITE (NBRC), Chiba Japan; 3) Oregon State University, Corvallis OR USA.

Through a community sequencing project with the US Department of Energy Joint Genome Institute, we sequenced genomes from three early diverging fungi including two zygomycetes, and *Gonapodya prolifera*, an aquatic species that grows on decaying plant material in stagnant water. We hypothesized that if fungi evolved in association with green algae from the land plant lineage, then their genomes would share fungus-specific genes for the breakdown of plant-specific polysaccharides such as the pectins that are known only from the Streptophytes, i.e. the land plants and their algal allies. Our aims were (1) to analyze patterns of expansion of pectinases among fungi and (2) to infer, based on patterns of pectinase gene expansion, the geological timing of association between plants and fungi. Analyzing the distribution of pectinases, the *Gonapodya* genome has 27 genes representing 5 of the 7 classes of pectin-specific enzymes known from fungi. Most of *Gonapodya*'s pectinases share a common ancestry with pectinases from Ascomycota and Basidiomycota. Indicating functional as well as sequence similarity, *Gonapodya* can use pectin as a carbohydrate source for growth in pure culture. Shared pectinases of Ascomycota, Basidiomycota and *Gonapodya* provide evidence that even ancient fungi were extracting nutrients from the plants in the

green lineage. This means that 750 million years, the estimated maximum age of origin of the pectin-containing streptophytes also represents a maximum age for the divergence of Chytridiomycota from the lineage including Dikarya.

The Chytrid secretome –a comparative analysis of the secretome of an aerobic, anaerobic and pathogenic Chytrid species. L. Lange[1], B. Pilgaard[1], A. Pedersen[2], F. Gleason[3], P. Busk[1]. 1) Aalborg University, Copenhagen Sv, Denmark; 2) Technical University of Denmark, Kongens Lyngby, Denmark; 3) University of Sydney, NSW, Australia.

This study focuses on the fungal secretome and builds on recent developments in genome sequencing and resolution of the phylogeny of the major fungal groupings. The secretome is biologically important as it reflects interaction; not just what the fungus is but how it grows and competes. Chytridiomycota is considered the earliest diverging fungal lineage of free living fungal species as the physiology of the earlier endoparasitic Cryptomycota has a life form where interaction is not well developed. Therefore Chytridiomycota are chosen to elucidate the composition of the most basal fungal secretome. The focus of the present study is a genome sequencing of the aerobic lignocellulose degrading chytrid, *Rhizophlyctis rosea.* The study includes mining of the genome for genes of secreted proteins; recombinant expression and characterization of a selected key enzyme, the GH45 cellulase; a description of the enzyme activity profile of the *R. rosea* secretome; and a characterization of the ecology and substrate association of the *R. rosea* isolate. The study further includes a comparison of the secretome composition of three very different chytrids: *R. rosea*, the sequenced anaerobic rumen chytrid *Orpinomyces* sp (Neocallimastigomycetes) and *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in amphibians. Highly significant differences are observed. The genomes compared are searched using a sequence analysis methodology (Peptide Pattern Recognition) developed by us (group of first author), which allows for alignment-free gene discovery and robust prediction of enzyme function from sequence. The current study includes analysis of how key substrate-degrading secretome enzymes (cellulases, hemicellulases, amylases, proteases) fit in phylogenetic trees, in an attempt to shed light on how these enzymes of the chytrid secretome have developed in perspective of similar developments in other fungal groups. The resulting phylogenetic trees are used as the basis for discussing the possible roles of mechanisms such as horizontal transfer, gene duplication and loss and convergent evolution.

The genomic landscape of early fungal evolution: Genomic innovations in the earliest fungal ancestors. Laszlo G Nagy[1], Robin A Ohm[2], Robert Riley[3], Francis M Martin[4], Igor V Grigoriev[3], David S Hibbett[5]. 1) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, HAS, 6726 Szeged, Hungary; 2) University of Utrecht, Department of Microbiology, 3584CH Utrecht, The Netherlands; 3) U.S. Department of Energy Joint Genome Institute, Walnut Creek, California 94598, USA; 4) INRA, UMR 1136, INRA-Nancy Université, Interactions Arbres/Microorganismes, 54280 Champenoux, France; 5) Clark University, Biology Department, Worcester, MA 01610, USA.

Fungi possess a plethora of genomic and phenotypic traits that make them unique and economically important among other life forms on Earth. Whereas vast knowledge has accumulated about many of these in extant model systems, the early evolutionary events that established generic fungal traits are hardly known. What are genomic events that lead to the emergence of a unique fungal lineage? What distinguishes fungi from closely related unicellular forms and animals? To address these questions, we reconstructed gene duplication-loss events in 59 fungal and outgroup species for which whole genome sequences have been published and inferred the ancestral genome composition of the last common ancestor (LCA) of Fungi and that of Dikarya. We reconstructed 1935 and 2794 gene duplications on the branches leading to the LCA of Fungi and the LCA of Dikarya, respectively. On the other hand, only 96 gene losses were inferred in the fungal LCA and 1182 in the Dikarya. Genomic innovations in the fungal ancestor are, among others, enriched in protein families related to intracellular transport and chitin metabolism, whereas those in the LCA of Dikarya are enriched in protein families showing oxidoreductase, ion transporter and chitin synthase activities. Notably, several domains of unknown function (DUF) were found to have emerged either in the LCA of Fungi and that of Dikarya, possibly marking as yet uncharacterized fungal-specific protein families. We discuss the results in the context of fungal functional diversification.

Comparative analysis of transcription factors families across fungal tree of life. Asaf Salamov, Igor Grigoriev. DOE Joint Genome Institute, Walnut Creek, CA.

Transcription factors (TFs) are proteins that regulate the transcription of genes, by binding to specific DNA sequences. We analysed the distribution and evolution of 60 known TF families in more than 300 fungal genomes from Mycocosm portal (http://jgi.doe.gov/fungi/). We have shown that while TF families, unique to fungal kingdom , like Zinc finger Zn2Cys6 and fungal-specific TFs, make up the largest fraction of TFs repertoire in most fungal genomes, especially greatly expanding in Pezizomycotina clade of Ascomycota, the universal eukaryotic TFs, like HLH, Homeobox, bZIP, GATA and others, are more abundant in Zygomycota and other early-divergent clades. We discuss the different evolutionary pathways of individual TF families.

Phylogenomics of the Zygomycete lineages: Exploring phylogeny and genome evolution. J E Stajich[1], G Benny[2], M Berbee[3], N Corradi[4], I V Grigoriev[5], A Gryganskyi[6], T Y James[7], A Kuo[5], K O'Donnell[8], R W Roberson[9], M Smith[2], J Spatafora[10], T N Taylor[11], R Vilgalys[12], M White[13]. 1) U California-Riverside, Riverside, CA; 2) U Florida, Gainesville, FL; 3) U British Columbia, Vancouver, BC CANADA; 4) U Ottawa, Ottawa, ON CANADA; 5) DOE Joint Genome Institute, Walnut Creek, CA; 6) Lambert Spawn, Coatesville, PA; 7) U Michigan, Ann Arbor, MI; 8) USDA ARS NCAUR, Peoria, IL; 9) Arizona State U,Tempe, AZ; 10) Oregon State U, Corvallis, OR; 11) U Kansas, Lawrence, KS; 12) Duke U, Durham, NC; 13) Boise State U, Boise, ID.

The Zygomycete lineages mark the major transition from zoosporic life histories of the common ancestors of Fungi and the earliest diverging chytrid lineages (Chytridiomycota and Blastocladiomycota). Genome comparisons from these lineages may reveal gene content changes that reflect the transition to nonflagellated, filamentous, and multicellular Dikarya (i.e., Ascomycota and Basidiomycota). The zygomycete lineages have been classified at times as a single monophyletic group and also split into an unresolved paraphyly. Phylogenomic analyses provide substantial support for two monophyletic clades one containing Entomophtheromycota,

Kickxellomycotina, and Zoopagomycotina (EKZ) and the other comprising Mortierellomycotina, Mucoromycotina, and Glomeromycota (MMG). Comparison of gene content among the species and their outgroups identified zygomycete-specific genes and confirmations of gene losses and gains that correspond to the transition from a zoosporic ancestor to primarily filamentous or yeast growth forms. Genome sequences from more than 35 zygomycetes were used to evaluate the phylogenetic position of these lineages, and compare gene content and phylogenetic relationships among thousands of groups of orthologous genes with animal and zoosporic fungal outgroups and the Dikarya fungi. The Zygomycete Genealogy of Life project is building a phylogenomic framework to address genomic and morphological evolution, broad genome and transcriptome sampling of zygomycete lineages including host-associated species, incorporating fossil-based dating, bioimaging, and improved descriptions of these fungi in the Encyclopedia of Life.

Genomic pathways key to intracellular mycoparasitism in the Rozellomycota C. Alisha Quandt[1], Daniele Corsaro[2], Rolf Michel[3], Nicolas Corradi[4], Timothy James[1]. 1) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA; 2) CHLAREAS Chlamydia Research Association, Nancy, France; 3) Laboratory of Medical Parasitology, Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany; 4) Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, ON, Canada.

The relationship between *Rozella allomycis*, an intracellular mycoparasite of *Allomyces*, and Microsporidia, intracellular parasites of animals, has been hypothesized for several years now. The nuclear and mitochondrial genomes of *Rozella allomycis* lack many of the basic genes for primary metabolism but have not undergone genome compaction to the extent seen in the multiple Microsporidia genomes sequenced. More recently, the discovery of diverse and seemingly ubiquitous cryptic fungi closely related to *R. allomycis*, has led to proposal of a single clade of early diverging fungi, called by various names (*e.g*. Cryptomycota, Rozellomycota, Opisthosporidia). Here, we present results from the genomes of Rozellomycota. We analyzed the transcriptional profile of *R. allomycis* growing endoparasitically in dual culture with its host, *Allomyces*, with analysis of the most highly expressed genes, genes involved in parasitism, and comparison with genes present in Microsporidia and other mycoparasitic fungi. We also analyzed the expression of nucleotide transporters in the *R. allomycis* genome which are known to have been horizontally transferred from chlamydia into the common ancestor of *Rozella* and Microsporidia. Because these genes are hypothesized to be involved in energy and nucleotide theft, we hypothesize they may have facilitated the evolution of intranuclear parasitism observed in newly described Rozellomycota such as the amoeba parasite, *Paramicrosporidium*.

Reduced fungal genomes of microsporidia: effects on key cellular processes. Cameron Grisdale, Thomas Whelan, Naomi Fast. Botany Dept, University of British Columbia, Vancouver, BC, Canada.

Microsporidia are obligate intracellular parasitic fungi that have some of the smallest and most compact eukaryotic genomes known. Effects of this extreme genome reduction are seen in all aspects of these parasites' biology, including effects on the ubiquitous eukaryotic processes of transcription and pre-mRNA splicing. The spliceosome is a large macromolecular machine that is conserved across eukaryotes and possesses 5 small nuclear RNAs (snRNAs) and hundreds of associated proteins. The spliceosome mediates splicing through interactions with short conserved sequences found in spliceosomal introns: the 5' and 3' splice sites and the branchpoint sequence. We report that the spliceosome of microsporidia is greatly reduced, with only ~30 recognizable protein components in *Encephalitozoon cuniculi*, a mammal-infecting microsporidian with a genome size of ~2.9Mbp. Indeed, *E. cuniculi* also appears to lack U1 snRNA, which plays an important role early in splicing by recognizing the 5' splice site. Although U1-independent splicing has been observed experimentally, it is not known to occur in nature. To assess whether the reduced nature of the spliceosome affects splicing efficiency, we carried out high-throughput transcriptome sequencing to assess the intron status of polyadenlylated transcripts in the intracellular stages of *E. cuniculi*. The *E. cuniculi* genome also has a reduced set of very short spliceosomal introns; the vast majority of the nearly 40 introns are less than 25 nt long. Our transcriptomic analysis indicates very low levels of splicing compared to other fungal lineages—in line with a reduced and, perhaps, inefficient spliceosome. However, 4 introns showed splicing levels above 75% and one intron merited further consideration. At 76 nt., it is almost 3 times longer than most microsporidian introns, and it possesses unusual sequence elements that we predict enhance pairing with the reduced microsporidian spliceosome. Furthermore, we have identified this unusual intron in multiple ORFs in diverse microsporidian genomes, where its distribution could also have implications for our understanding of intron gain. Overall, these results highlight the potential regulatory role for splicing in these reduced fungi.

Genome analyses reveal evidence for polyploidy and recent clonal expansion in the microsporidian honey-bee pathogen *Nosema ceranae*. Adrian Pelin, Mohammed Selman, Nicolas Corradi. Department of Biology, University of Ottawa, Ottawa, Ontario, Canada.

Nosema ceranae is a microsporidian pathogen whose infections have been associated with recent global declines in the populations of western honey bees (*Apis mellifera*). Despite the potential economic and ecological threat that *N. ceranae* may represent, many aspects of its biology, including their mode of reproduction, propagation and ploidy, are poorly understood. Here, we set to acquire knowledge into these biological aspects by re-sequencing the genome of 9 isolates of this species harvested from 9 geographically distant beehives, and by investigating their level of polymorphism. Our analyses uncovered the presence of a large genetic diversity within all isolates, but very little hive-specific polymorphism. The nature, location and distribution of genetic variation we identified suggest that each beehive is infected by a population of *N. ceranae* cells that are least polyploid (4n or more) and predominantly clonal (i.e. sex is rare). Finally, several phylogenetic analyses based SNP data extracted from these parasites and their hosts failed to support their current geographical structure, suggesting that the isolates we analysed are unlikely to have reached their current geographical areas through natural means.

Friday, March 20 3:00 PM–6:00 PM
Nautilus

Environmental Metagenomics
Co-chairs: Rytas Vilgalys and Cheryl Kuske

Comparative metatranscriptomics of soil fungal and bacterial communities in temperate forests and arid grasslands. Cedar N. Hesse[1], Blaire Steven[1,2], LaVerne A. Gallegos-Graves[1], Cheryl R. Kuske[1]. 1) Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM; 2) The Connecticut Agricultural Experiment Station, New Haven, CT.

The direct measurement of expressed genes from natural microbial communities has only recently been made possible by advances in sample preparation methods and next-generation sequencing. Environmental metatranscriptomes enable the exploration of actively transcribed genes from taxonomically complex natural environments, such as soil or decaying plant matter. Here we present a comparative analysis of three metatranscriptomic studies from three different temperate biomes: cyanobacterial biocrusts from Western U.S. arid grasslands, decomposing leaf-litter from a northern hardwood forest, and multiple soil strata from a temperate pine forest. All samples were prepared, sequenced, and analyzed in a similar fashion thereby facilitating accurate comparison. While these ecosystems are drastically different from one another in many ways including their resident soil microbial community, geochemistry, and climate, we can show common signatures of microbial gene expression across all sites. Conversely, the differences in metatranscriptomic profiles can largely be explained by the underlying taxonomic differences among sites. Here we present an overview of our metatranscriptome sequencing and analysis techniques and identify distinguishing features of each study site.

Metatranscriptomic analysis of ectomycorrhizal functioning in pine forest soils. Hui-Ling Liao[1], Y Chen[2], T.D. Bruns[3], K.G. Peay[4], J.W. Taylor[3], S. Branco[3], J.M. Talbot[5], R. Vilgalys[1]. 1) Biology Dept, Duke Univ, Durham, NC; 2) Medicine Dept, Duke Univ, Durham, NC; 3) Plant and Microbial Biology Dept. UC Berkeley, Berkeley, CA; 4) Biology Dept, Stanford Univ, Stanford, CA; 5) Biology Dept, Boston Univ, Boston, MA.

Metagenomic approaches that assess coordinated genetic activities within complex soil microbial community show great promise for identifying key functions of different microbes in natural soil including conversion of carbon and the regulation of plant access to nitrogen and phosphorus. These studies are providing new insight into fungal functioning within natural forest soil ecosystems. Here we report a method for metatranscriptomic analysis of interactions between ectomycorrhizal fungi (EMF) and their pine hosts from native and experimental forest soil systems. Our protocol relies on poly-A enrichment of plant and fungal transcripts from soil and mycorrhizal root clusters. Using advanced computational workflows involving de-novo assembly, mapping and blastX, we were able to identify a variety of expressed genes from soil, including 0.3% from fungal rRNA, 0.5% from 16S rRNA, ~3% from individual highly expressed gene groups respectively (e.g. G-protein, protease, etc); 0.5 to 3% from individual nutrient degradation enzymes (e.g. glucosidase, amidase, urease, phosphatase, sulfatase, laccase, chitinase, other hydrolases, etc), 0.05 to 2% from the individual gene groups that regulate microbial development and communication (e.g. transport, cell signaling, iron carrier, etc). Using soil metatranscriptomics, the activities of many dominant fungal taxa could be identified, with over 30% of reads assigned to specific functions of individual fungal taxa. These approaches also allowed us to analyze nutrient cycling gene expression patterns for conserved and unique soil enzymes at a fine dimension scale.

Turning the black box inside out: inferring the taxonomic and functional diversity of mycorrhizal fungi through the use of amplicon, metagenomic, and metatranscriptomic sequencing. Joshua R Herr. Michigan State University, East Lansing, MI.

It has long been understood that fungi contribute to many key ecosystem processes. This is particularly important in soils where fungi are the main drivers of plant organic matter decomposition and facilitate nutrient uptake for their host plants, thereby affecting plant growth and fitness. Additionally, fungi contribute to carbon sequestration, shape seedling establishment, and evidence suggests that mycorrhizal fungi may contribute to the distribution of carbohydrates from one plant to another, possibly regulating the survival of nurse seedlings. Despite their important ecological roles, there is a paucity of information regarding taxonomic and functional diversity. This is due largely to the fact that most fungi are unculturable, lack known sexual structures, and are known only by nucleotide identification. Next-generation sequencing technologies have revolutionized the ability to use sequence data to address ecological and physiological questions, and mycorrhizal fungi are not immune to these advances. By attempting to integrate amplicon, whole genome, and metatranscriptome sequencing experiments in a single forest plot, a more complete picture of taxonomic and functional diversity can be gained than by using any one type of data alone. Using publically available large datasets and by sifting through fungal nucleotide sequences derived from soil metagenomes, I provide a framework for taxonomic and functional diversity of fungi associated with plant roots and discuss the challenge of trying to integrate data from genomic and marker gene sequencing to make conclusions regarding diversity.

Trancriptomics and spectroscopy provide novel insights into the mechanisms of litter decomposition by ectomycorrhizal fungi. Anders Tunlid. Dept Biology, Lund University, Lund, Sweden.

Globally, soil organic matter (SOM) stores more carbon (C) than is present in the terrestrial biomass and the atmosphere combined. A large portion of the SOM is present in temperate and boreal forests. Whether this pool will capture, store or release C is highly dependent on the activity of microorganisms that decompose SOM. Traditionally, filamentous, saprotrophic fungi are thought to have a unique ability to degrade SOM including lignocellulose and they are considered to be the main decomposer of forest SOM. By contrast, biotrophic fungi, such as ectomycorrhizal (ECM) fungi are thought to have only limited capacity to decompose complex SOM. This view is supported by

genome sequencing showing that ECM have lost many of the genes that encode hydrolytic plant cell wall-degrading enzymes in their saprotrophic species. Nevertheless, by using spectroscopic analyses and transcriptome profiling, we have demonstrated that the ectomycorrhizal fungus *Paxillus involutus* have a significant capacity to decompose organic matter when acquiring nitrogen from plant litter. The observed chemical changes were consistent with a hydroxyl-radical attack, involving Fenton chemistry similar to that of saprophytic brown-rot fungi. Further experiments showed that the decomposition of plant litter and assimilation of nitrogen in *P. involutus* are triggered by the addition of glucose, while the addition of ammonium, the most abundant inorganic N form in forest soils, had relatively minor effects on the decomposing activity. Taken together, these experiments suggest that at least some ECM fungi can decompose SOM using an oxidative mechanism present in brown-rot fungi. We propose that the primary function of the decomposing activity is not to assimilate the released C, rather to mobilize the organic N that is embedded in recalcitrant SOM complexes. The released C may either be further degraded by saprotrophic microorganisms or sequestered in stable SOM-mineral aggregates. The prospects of using spectroscopic methods and transcriptomic data to identify specific transcripts or chemical signatures that can be used as biomarkers for probing the decomposing activity of soil-living fungi will be discussed.

The interactomes of competeing fungi during wood decomposition succession. Daniel C Eastwood[1], Suzy Moody[1], Jennifer Hiscox[2], Melanie Savoury[2], Ed Dudley[1], Hilary Rogers[2], Carsten Muller[2], Lynne Boddy[2]. 1) Swansea University, Swansea, United Kingdom; 2) Cardiff University, Cardiff, United Kingdom.

Wood decomposition is a critical process in nutrient recycling within forests systems and has wider implications for the global carbon cycle. Decay is driven predominanly by Agaricomycete fungi that have specialised to breakdown recalcitrant lignocellulose. Fungi must balance decomposition and substrate utilisation with continuing growth and foraging, sporulation and competition. The outcomes of competitive interactions are varied with some species exhibiting a more agressive growth than others. Interaction outcomes are also influenced by physical parameters, such as temperature and water availability, and the potential impacts of climate change on these systems is unknown. This ongoing study investigates the transcriptomic and proteomic responses of intermingling and competing decay fungi growing through beech wood blocks during a time course and under different physical conditions.

Specific expression of candidate effectors of the rust fungus *Melampsora larici-populina* during infection of its two host plants, larch and poplar. Sebastien Duplessis[1,2], Stephane Hacquard[1,2,3], Antoine Persoons[1,2], Christine Delaruelle[1,2], Jeremy Petrowski[1,2], Pascal Frey[1,2], Benjamin Petre[1,2,4]. 1) INRA, UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, 54280 Champenoux, France; 2) Université de Lorraine, UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, 54280 Champenoux, France; 3) Present address, Max Planck Institute for plant breeding research, Köln, Germany; 4) Present address, The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.

Melampsora larici-populina (Basidiomycete, Pucciniales) is one of the rust fungi responsible for the poplar leaf rust disease. It has a complex macrocyclic and heteroecious life cycle, marked by the production of five different spore forms in two different host plants: larch (sexual reproduction) and poplar (asexual clonal reproduction). The asexual stage leads to severe rust epidemics recorded in poplar plantations. As for other rust fungi, the asexual stage has been well covered and characterized, whereas we have almost no knowledge of other stages of the life cycle. Particularly, the capacity for the fungus to infect hosts with distinct taxonomical positions raises questions about the molecular bases underlying the specificity of host-rust interactions. Following the genome sequencing of *M. larici-populina* isolate 98AG31, the secretome annotation has revealed a large repertoire of nearly 1200 genes encoding small secreted proteins and expression profiles of these candidate rust effectors have been defined by oligoarray-based transcriptomics during poplar infection. We present here the transcriptome analysis by RNA-Seq (Illumina) of three fungal structures associated to the infection and reproduction stage on larch: basidia, spermogonia and aecia. These were obtained following an original system for controlled infection in laboratory conditions using the reference isolate 98AG31. Almost 300 millions reads were generated for each condition (three biological replicates) and were used to show specific expression profiles in the larch host. Comparison with the profiles previously obtained in poplar reveals the presence of specific sets of candidate effectors expressed either in each or in both host plants.

***Epichloë* fungal endophytes and the formation of synthetic symbioses in Hordeeae (=Triticeae) grasses.** Richard Johnson[1], Marty Faville[1], Milan Gagic[1], Paul Maclean[2], Wayne Simpson[1], Linda Johnson[1]. 1) Forage Improvement, AgResearch Grasslands, Palmerston North, New Zealand; 2) Knowledge & Analytics, AgResearch Ruakura, Hamilton, New Zealand.

Epichloë (formerly including the genera *Epichloë* and *Neotyphodium*) are grass-colonising fungi, belonging to the family Clavicipitacea, that infect grasses within the subfamily Pooideae including some within the tribe Hordeeae. These fungi produce a number of secondary metabolites in their host plants that can be of benefit in agricultural systems, as afforded by *Epichloë* endophytes in Poeae grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*). There have been no accounts of modern domesticated Hordeeae hosting *Epichloë* endophytes, but there have been reports in *Elymus*, *Hordeum* and other wild grasses within the tribe. We have screened over 1000 seed accessions worldwide of *Elymus* and *Hordeum* and have characterised over 100 genetically distinct *Epichloë* strains from these wild populations. Inoculation of modern cereals such as wheat and rye indicates that a range of host-endophyte compatibility outcomes are possible, ranging from incompatible (stunted plants) to fully compatible symbioses. Further to this we have demonstrated that both endophyte strain and host genotype influence the compatibility outcome. To understand the molecular determinants of incompatible versus compatible host-endophyte associations we have performed transcriptomics experiments (RNA-Seq) on a range of endophyte-host combinations with different compatibility outcomes. A number of host defence related pathways have been identified during incompatible associations and fungal differentially expressed genes are enriched for small secreted proteins (putative effectors). Bioinformatics analysis of several different *Epichloë* genomes has shown that the number of these putative effectors differs significantly between strains with some being unique to a particular strain. Knowledge of these host and endophyte factors will guide us in our aim to create fully functional cereal-endophyte associations.

Fungal-bacterial interactions are mediated by fungal lipid signaling and a common set of bacterial factors. O. Lastovetsky[1], S. Mondo[2], T. Pawlowska[2]. 1) Graduate Field of Microbiology; 2) School of Integrative Plant Science, Cornell University, Ithaca, NY.

Fungal-bacterial symbioses are an emergent field of study, and currently little is known about how they are established and maintained. In particular, the genetic basis for interaction between fungi and bacteria is poorly understood. We employ the association between the fungus *Rhizopus microsporus* and the endosymbiotic bacterium *Burkholderia* sp. as a model to identify genes involved in fungal-bacterial interaction and symbiosis. Within the *R. microsporus* species there are isolates that harbor endobacteria (*host*) and there are naturally endobacteria-free isolates (*non-host*). We analyzed fungal and bacterial gene expression during compatible (host with endobacteria) and incompatible (non-host with endobacteria) interactions. This analysis identified dramatic transcriptional changes in the host in response to its bacterial endosymbiont (>750 genes), as compared to the non-host (48 genes). Notably, genes involved in receptor signaling, actin rearrangement and lipid metabolism were overexpressed in the host. In both fungi, responses to bacteria converged on the production of two lipid signaling molecules – diacylglycerol (DAG) and phosphatidic acid (PA). While DAG and PA are interconvertible in eukaryotic cells, they control different pathways. Gene expression in the host fungus pointed to maintaining higher levels of PA over DAG, and the opposite occurred in the non-host. We speculate that maintaining higher levels of one versus the other controls the establishment of symbiosis. Analysis of bacterial transcriptomes showed that bacteria responded to both host and non-host fungi in very similar ways. This allowed for the identification of a common set of mechanisms that bacteria use for interaction with fungi. These included Type 3 Secretion System and its effectors, capsular polysaccharides and a 2-component regulatory system. Interestingly, these mechanisms are also known to be important for the interaction between bacteria and other eukaryotic hosts such as plants and animals. We thus showed that bacteria possess a common set of mechanisms for interaction with plants, animals and fungi.

Friday, March 20 3:00 PM–6:00 PM
Scripps

Fungal Biotechnology
Co-chairs: Kazuhiro Iwashita and Randy Berka

Fungal artificial chromosomes for mining of the fungal secondary metabolome. J. Bok[1], J. Albright[2], R. Ye[3,4], D. Mead[4], M. Wagner[4], A. Krerowicz[4], A. Goering[5], K. Clevenger[5], T. Velk[1], P. Thomas[5], N. Kelleher[2,5], N. Keller[1], C. Wu[3,4]. 1) Department of Medical Microbiology & Immunology, University of Wisconsin, Madison, Wisconsin, USA; 2) Department of Chemistry, Northwestern University, Evanston, Illinois, USA; 3) Intact Genomics, Inc. St Louis, Missouri, USA; 4) Lucigen Corporation, Middleton, Wisconsin, USA; 5) Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois, USA.

With thousands of fungal genomes being sequenced, each genome containing up to 70 secondary metabolite (SM) clusters 30 - 80 kb in size, breakthrough techniques are needed to characterize this SM wealth. Here we describe a novel system-level methodology for unbiased cloning of intact SM clusters from a single fungal genome for one-step transformation and expression in a model host. All 56 intact SM clusters from *Aspergillus terreus* were individually captured in self-replicating fungal artificial chromosomes (FACs) containing both *E. coli* F replicon and an *Aspergillus* autonomously replicating sequence (AMA1). Candidate FACs were successfully shuttled between *E. coli* and the heterologous expression host *A. nidulans*. As proof-of-concept, an *A. nidulans* FAC strain was characterized in a novel liquid chromatography-high resolution mass spectrometry (LC-HRMS) and data analysis pipeline leading to the discovery of the *A. terreus* astechrome machinery.

Development of a community consensus model for *Aspergillus niger*. Julian Brandl[1], Mhairi Workman[1], Mikko Arvas[2], Vera Meyer[3], Mikael R. Andersen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) VTT Technical Research Centre of Finland, Finland; 3) Department of Applied and Molecular Microbiology, Institute of Biotechnology, Technical University of Berlin, Germany.

Fungal primary metabolism is an essential part of fungal physiology and affects all phenotypic traits of the organism as well as carrying the biotechnological potential for the fungal host. While the study of individual pathways have gained essential knowledge and important scientific breakthroughs, a genome-scale view of metabolism is required to gain a holistic understanding of the cell. Mathematical models based on the stoichiometry of known enzymatic reactions have been developed in order to facilitate this approach and proven useful for guiding metabolic engineering in well characterized model organisms like *S. cerevisiae* and *E. coli.* With the sustained interest in *Aspergillus niger* as a potent host organism for citric acid and enzyme production, it is timely to improve on previous genome-scale modeling efforts. Here we aim at updating the genome-scale model by a combination of experimental work and integration of published information. This joint effort of international collaborators and our group will yield a community-consensus model of the *Aspergillus niger* metabolism. In order to improve the gene assignments contained in the current version of the model, we will use comparative genomics to identify shared isoenzymes and gene groups between closely related species in the section *Nigri*. To accurately examine and model the catabolic potential of the fungus, we will apply Biolog plates for the screening of more than 270 carbon and nitrogen sources. This knowledge will aid to identify missing pathways in the model and validate the presence of many pathways already included. Additionally the biosynthesis of 2-300 secreted enzymes will be included in the new version of the model. In conclusion this project aims at generating an experimentally validated community-consensus model of the *A. niger* metabolism being able to describe and predict beneficial modifications to the metabolic network in order to improve protein production on a variety of different substrates.

Inducing sexual reproduction in the industrial fungus *Aspergillus oryzae*: Can the domesticated fungus get sexy again? Jun-ichi Maruyama, Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

Aspergillus oryzae is an industrially important fungus used for the traditional fermentative manufacture of Japanese foods and heterologous protein production. As sexual reproduction has not been observed in *A. oryzae*, it is quite difficult to breed strains with industrially useful characteristics. We identified two mating types of *A. oryzae* (MAT1-1 and MAT1-2)[1)], indicating that this fungus has the potential for sexual reproduction in a heterothallic manner.

Cell fusion is the first process in sexual reproduction, and we have recently demonstrated that *A. oryzae* can enter cell fusion[2,3)]. The second important step is the formation of sexual reproductive structures. A sclerotium, a survival mycelial structure, is capable of acting as repositories for ascocarps forming sexual ascospores in other *Aspergillus* species. However, *A. oryzae* is thought to have been domesticated from the ancestor *Aspergillus flavus*, and *A. oryzae* strains have lost the ability to form sclerotia or have a much lower ability.

We deleted the *ecdR* gene encoding for a transcription factor negatively regulating sclerotia formation[4)], which increased heterokaryotic sclerotia of the two *A. oryzae* mating-type strains. However, this was not sufficient to induce the formation of sexual reproductive structures. Then, sexual reproduction-related genes were overexpressed in the Δ*ecdR* strain, and ascocarps, asci and ascospore-like structures were formed in the sclerotia. Thus, these sexual reproductive structures were found for the first time in *A. oryzae*. If genetic crossing is found in the ascospore-like structures, it will be possible to perform crossbreeding for industrially useful strains in *A. oryzae*.

1) Wada *et al.* (2012) *Appl. Environ. Microbiol.* 2) Tsukasaki *et al.* (2014) *Biosci. Biotechnol. Biochem.* 3) Wada *et al.* (2014) *Appl. Microbiol. Biotechnol.* 4) Jin *et al.* (2011) *Fungal Genet. Biol.*.

Codon optimization increases mRNA levels of heterologous genes in *Aspergillus* fungi. Katsuya Gomi, Mizuki Tanaka. Grad Sch Agric Sci, Tohoku Univ, Sendai, Japan.

Filamentous fungi, particularly *Aspergillus* species, have recently attracted attention as host organisms for recombinant protein production. The secretory yields of heterologous proteins are generally low compared with those of homologous proteins or proteins from closely related fungal species. Among a lot of strategies to produce substantial amounts of recombinant proteins, codon optimization is a powerful tool for improving the production levels of heterologous proteins. Although there were several reports that codon optimization causes an increase in the steady-state mRNA levels of heterologous genes in filamentous fungi, the mechanism that determines the low mRNA levels when native heterologous genes are expressed was poorly understood. We recently showed that the transcripts of heterologous genes are polyadenylated prematurely within the coding region and that the heterologous gene transcripts can be significantly stabilized by codon optimization, which is possibly attributed to the prevention of premature polyadenylation in *Aspergillus oryzae*. Thus, codon optimization results in an increase in the amount of mRNA transcripts, which can lead to improve the production level of heterologous proteins in filamentous fungi including *Aspergilli*.

Fast forward genetics to understand fungal enzyme secretion. Scott Baker[1,2], Kevin McCluskey[3], Blake Simmons[2,4], Jed Lynn[1,2], John Gladden[2,4], Jon Magnuson[1,2], DOE Joint Genome Institute. 1) Pacific Northwest Natl Lab, Richland, WA; 2) DOE Joint Bioenergy Institute, Emeryville, CA; 3) Fungal Genetics Stock Center, Kansas State University, Manhattan, KS; 4) Sandia National Laboratories, Livermore, CA.

Filamentous fungi are economically important platforms for industrial cellulase production. These organisms possess the ability to secrete an assortment of enzymes that deconstruct plant biomass and other complex nutrient substrates. High enzyme secretion fungal mutants have been generated over the last several decades in various ascomycete species. We have analyzed the “resequenced” genomes of multiple strains from three different species possessing mutant enzyme secretion phenotypes in order to generate a mechanistic understanding of the biological pathways that mediate biomass deconstruction in fungi. Our analyses indicate that a small group of orthologous genes play key roles in secretome regulation.

Enhancement of plant performance with synergistic endophytic plant symbionts. Gary Harman, Molly Cadle-Davidson, Walid Nosir. R&D, Advanced Biological Marketing, Geneva, NY.

In the past decade, specific strains of unrelated root-colonizing microorganisms have been shown to be endophytic plant symbionts. These organisms typically grow and proliferate in the outer layers of the root cortex. Strains of diverse endophytes have similar qualitative effects on plants and induce system-wide changes in plant gene expression that alter plant physiology and result in numerous benefits. Some of these beneficial effects are enhanced photosynthetic rates/efficiencies, improved resistance to biotic and abiotic stresses, improved nitrogen and other nutrient use efficiencies, and improved seed/seedling performance. These changes occur across a wide range of plant species including monocots and dicots and are very similar between different genera of microorganisms. Organisms that provide these benefits include some *Bacillus* and Pseudomonads, various Rhizobia species, mycorrhizal fungi, and free-living fungi in the genera *Trichoderma* and *Piriformaspora*. In many cases the effects result in improved root formation, growth and increased crop yields. When effectively harnessed, the ability to establish this endophytic, symbiotic relationship holds remarkable potential for all horticultural and agricultural production systems. ABM is investing in the discovery, characterization and leveraging of mechanisms underlying these microbe-plant interactions and their downstream effects. Proteomic, genomic, and high throughput phenotyping methods are being applied to the plant-beneficial microbe collections developed at Cornell and ABM over the last 40 years (~40,000 strains). The powerful genetics behind these collections facilitate application of cutting-edge, modern techniques to materials that have been thoroughly characterized previously via brute force methods necessary at the time the collections were made. These approaches have resulted in the definition of

plant pathways and microbial metabolites key in the trigger and subsequent phenotypic expression of traits associated with beneficial microbes.

High-level production of mono-component and enzyme mixtures in *Myceliophthora thermophila*. Cristina Llavata Peris, Jurian Bronkhof, Jurgen Snelders, Hans Visser, Jaap Visser, Jean-Paul Meijnen. Dyadic Netherlands, Wageningen, Netherlands.

The thermophilic fungus *Myceliophthora thermophila* C1 was developed into an efficient and versatile platform for high-level production of industrially relevant enzymes. By means of strain development strategies, such as random mutagenesis and targeted disruptions, we obtained two strain lineages that are being developed and exploited as enzyme production hosts. One strain lineage (HC-strains) is able to produce and secrete high amounts of enzyme mixtures that contain large amounts of (hemi-) cellulases. The other strain lineage (LC-strains) is impaired in its cellulase producing capability, resulting in low background-protein production. For that reason the LC-strains could be very suitable for the production of individual enzymes.

The LC strain has been further developed for high-level production of homologous enzymes. An open reading frame encoding an endoglucanase was introduced at multiple copies by multiple rounds of transformation. After fermentation optimization, protein levels of up to >25 g/L were reached of which ~ 80% was the overexpressed endoglucanase. These results indicate that the LC strain is capable of producing mono-component enzymes at high levels in a relatively pure form.

By transforming the LC strain with selected C1 genes, a wide collection of strains was obtained, each of which produced mainly one enzyme. This has ultimately led to an enzyme library of over 100 functional enzymes of which many have been purified and characterized in detail.

In conclusion, *M. thermophila* C1 was developed into a high-level protein-production platform. The HC strain is successfully applied to produce enzymes for the production of biofuels and biobased-chemicals. The LC strain is being used to produce single enzymes and defined combinations of enzymes. The obtained C1-enzyme library is a rich source for academic and industrial research. The properties of *M. thermophila* C1 make this fungus a highly suitable alternative for traditional fungal protein production hosts.

Enhanced Hydrolysis of Lignocellulosic Material. Taija Leinonen[1], Kristiina Järvinen[1], Susanna Mäkinen[1], Kari Juntunen[1], Alexandra Komander[2], Kim Langfelder[2], Jari Vehmaanperä[1], Terhi Puranen[1]. 1) Roal Oy, Rajamäki, Finland; 2) AB Enzymes GmbH, Darmstadt, Germany.

Limited resources of fossil fuels have raised a need for using biomass as a renewable and clean source of energy. One promising, alternative technology is the production of biofuels i.e. ethanol from (ligno)cellulosic materials. Enzymatic hydrolysis is considered as potential method for converting cellulosic biomass into fermentable sugars, and efforts have been made to improve the efficiency of the hydrolysis process. An effective enzyme mixture for cellulosic biomass hydrolysis contains at least cellobiohydrolases (CBH), endoglucanases (EG), beta-glucosidases (BG) and xylanases (XYN). The hydrolysis performance could be increased by optimization of the mixture components, or by improving the individual enzyme components in the mixture by protein engineering or enzyme discovery, or by adding novel auxiliary enzymes with additional activities into the mixture. In this work, *Acremonium thermophilum* CBHI, EG_A, EG_B and *Melanocarpus albomyces* FAE_B were cloned and produced in *Trichoderma reesei*. In the biomass hydrolysis experiments different combinations of enzymes were tested on variety of (ligno)cellulosic substrates. Obtained data indicate that by replacing the existing enzyme components of the mixture with the *A. thermophilum* CBHI, EG_A or EG_B and adding *M. albomyces* FAE_B as auxiliary enzyme into the mixture, have enhancing effect on hydrolysis yield.

Saturday, March 21 2:00 PM–5:00 PM
Merrill Hall

Impact of Fungal Metabolism on Pathogenicity
Co-chairs: Alistair Brown and Gustavo Goldman

Fungi challenge global food security and plant ecosystem health. Sarah Gurr, Dan Bebber. Biosciences, Univ Exeter, Exeter, United Kingdom.

Fungal diseases have been increasing 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). Indeed, 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).

This presentation will highlight some of the more notable persistent fungal and oomycete plant diseases of our times. It will draw attention to the emergence of new pathotypes affecting crop yields and to fungi and oomycetes decimating our natural and managed landscapes. I shall review some of our recent work looking at the movement of fungi polewards in a warming world (Bebber, Ramatowski and Gurr, 2013 *Nature Climate Change* 11 985), at the global distributions of crop pests and pathogens (Bebber *et al., 2014 New Phytologist* DOI:

10.1111/nph.1272) and of fungi *(*Bebber and Gurr 2014, *Fungal Genetics and Biology* DOI: .org/10.1016/j.fgb.2014.10.012) and at the saturation rate of crops by such organisms (Bebber, Holmes, Gurr, 2014 *Global Ecology & Biogeography* DOI: 10.111 /geb12214).

I shall conclude with some thoughts on the emergence of fungi in natural and crop ecosystems, of future threats and challenges and disease mitigation.

What is hemibiotrophy? Metabolic and mechanistic insights from gene function studies in the rice blast fungus *Magnaporthe oryzae*. Richard A. Wilson. Department of Plant Pathology, University of Nebraska at Lincoln, Lincoln, NE.

The hemibiotroph *Magnaporthe oryzae*, cause of the most serious disease of cultivated rice, colonizes leaf cells as a symptomless biotroph before entering its destructive necrotrophic phase. During biotrophy, *M. oryzae* is surrounded by a plant-derived extra-invasive hyphal membrane (EIHM) and remains undetected in the host while acquiring nutrients and growing cell-to-cell. What metabolic strategies facilitate *in planta* development and rapid host colonization are not well understood. To address this knowledge gap, our work has coupled live-cell imaging with gene function analysis to show that during early infection, *M. oryzae* readily metabolizes glucose through the pentose phosphate pathway and glycolysis in order to i) provide NADPH to fuel antioxidation and ii) generate sufficient ATP to signal biotrophic growth via cell cycle regulation. In contrast, at least some plant sources of nitrogen - such as purines and methionine - are not accessible to *M. oryzae* during biotrophy despite being present in the rice cell at concentrations that support fungal growth. Thus, during early rice infection, *M. oryzae* is adapted to thrive in glucose-rich, nitrogen-poor environments. We have hypothesized that this is the result of a trade-off between the nutrient demands of the fungus on the one hand, and the need to maintain the nitrogen-impermeable EIHM for plant defense suppression on the other. We sought to shed light on this conjecture by focusing on the relationship between hemibiotrophy and nitrogen metabolism. Here, we show how the ability to scavenge and assimilate ammonium under nitrogen-limiting conditions is essential for biotrophic growth but later, as symptoms develop, nitrogen metabolism switches to amino acid acquisition under perceived nitrogen-rich conditions. This suggests a fundamental shift in available nitrogen quantity and quality as biotrophy progresses into necrotrophy, and the resulting regulatory responses by *M. oryzae* are discussed. Together, our work provides a window on the dynamic metabolic landscape of blast disease (from the perspective of the fungus) and enriches our understanding of the molecular mechanisms governing *M. oryzae* growth in rice cells.

The cellulase specific transcriptome of *Trichoderma reesei* as influenced by light, photoreceptors and CRE1. Eva Stappler[1], Christoph Dattenböck[1], Doris Tisch[2], André Schuster[2], Monika Schmoll[1]. 1) Health & Environment, Bioresources, AIT - Austrian Institute of Technology, Tulln, Austria; 2) Institute of Chemical Engineering, TU Wien, Vienna, Austria.

Trichoderma reesei is adapted to degradation of plant cell walls and produces an efficient enzyme cocktail for this task. We investigated two regulation levels of the transcriptome of *T. reesei*: light and carbon source dependent control. We evaluated regulation by light, induction specific regulation and relevance of photoreceptors, a potential relevance of surface sensing in cellulase regulation and genes associated with repression of cellulase gene expression. We show that the carbon source (cellulose, lactose, sophorose, glucose, glycerol) is the major source of variation, with light having a modulating effect. 206 genes are significantly regulated in response to light across all carbon sources. 907 genes were specifically regulated under inducing conditions in light and 947 genes in darkness with 530 genes overlapping (1324 in total). In this gene set, significant enrichment was detected for functions in C-compound and carbohydrate metabolism, amino acid metabolism, energy and respiration. Evaluation of genomic distribution of genes regulated by light on cellulose, showed considerable overlap with previously described CAZyme clusters. Of the 1324 genes regulated specifically under inducing conditions in light, darkness or both, only 218 genes were found to be induction specific independent of light and not regulated by the photoreceptors. 282 of those 1324 genes were found to be regulated by CRE1 under inducing conditions. Comparison of gene expression upon growth on soluble (lactose, sophorose) and insoluble (cellulose) inducing carbon sources suggests the operation of a sensing mechanism for solid substrates, putatively involving G-protein coupled receptors, which regulates auxiliary proteins such as CIP1, CIP2 and swollenin as well as a hydrophobin gene. In order to evaluate the relevance of nutrient sensing for cellulase gene expression, we selected 9 GPCRs for functional analysis and 5 of them were found to be relevant for cellulase expression.

A synthetic biology approach for unveiling the ecologically-relevant secondary metabolites in phytopathogens. Yit-Heng Chooi, Peter Solomon. Research School of Biology, Australian National University, Acton, ACT, Australia.

Secondary metabolites (SMs) are known to play important roles in the virulence and adaptation of fungal phytopathogens. The genome of the major wheat pathogen, *Parastagonospora nodorum*, harbors 38 SM gene clusters. An ecological genomics approach was used to narrow down candidate SM gene clusters of potential pathogenic or ecological importance. A combination of reverse genetics and synthetic biology approaches were used to tease out the molecules encoded by these candidate clusters. Among them is a *P. nodorum* polyketide synthase (PKS) gene (*SN477*) that was highly expressed during wheat leaf infection. Heterologous expression in yeast demonstrated that *SN477* encodes the production of (*R*)-mellein. Whilst mutants of *P. nodorum* unable to synthesize (R)-mellein remained pathogenic, exposure of the compound to wheat seeds inhibited the germination. Another *P. nodorum* SM cluster harboring the PKS gene *SN8614* was up-regulated during the transition from necrotrophy to saprotrophy stage *in planta*. The overexpression of a transcriptional factor in the gene cluster activated the expression of this pathway in culture medium producing a red pigment not found in wild-type. This pigment caused significant necrosis on wheat leaves in a light-dependent manner. The compound structure was established to be a perylenequinone named elsinochrome C. This is the first report of elsinochrome C in *P. nodorum*. . We are currently reconstructing the whole pathway to understand the biosynthesis of this light-activated toxin. Finally, we were able to identify the PKS gene (*SN15829*) that encodes the production of the mycotoxin alternariol in *P. nodorum.* This was confirmed by both reverse genetics and heterologous expression of the PKS gene in *Aspergillus nidulans*. The *P. nodorum* alternariol PKS gene is different from that reported in *Alternaria alternata* previously.

The identification of *P. nodorum* alternariol PKS gene will facilitate the detection of alternariol-producing fungi in the environment. In conclusion, synthetic biology can be a promising tool for linking ecological genomics with chemical ecology.

Metabolism affects *Candida albicans* pathogenicity at multiple levels. Alistair Brown[1], Gordon Brown[1], Mihai Netea[2], Neil Gow[1]. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; 2) Nijmegen and Radboud Center for Infectious Diseases, Geert Grooteplein Zuid 8, Nijmegen, The Netherlands.

Metabolism is integral to the pathogenicity of the major fungal pathogen of humans, *Candida albicans*, influencing virulence at multiple levels. This fungus tunes its nutrient assimilation and metabolism to optimize growth and colonization of diverse niches in the host. This metabolic adaptation is also integrated with the expression of key virulence factors, it affects the susceptibility of *C. albicans* to the stresses is experiences in these host niches, and it modulates the resistance of this pathogen to antifungal drugs. Furthermore, metabolic adaptation modulates the vulnerability of *C. albicans* to innate immune defences. These effects are controlled by complex regulatory networks that link metabolism, morphogenesis, stress adaptation and cell wall remodelling. Together, these processes influence commensalism and infection. Consequently, current views of *Candida*-host interactions should be extended to include the impact of metabolic adaptation upon pathogenicity and immunogenicity.

Phenotypic switching in *Candida albicans*: towards integrating environmental inputs and cellular outputs. Iuliana Ene, Richard Bennett. Molecular Microbiology and Immunology, Brown University, Providence, RI.

The ability to switch between different phenotypic states is crucial for the success of *Candida albicans* as a human pathogen. While the white-opaque switch modulates mating, the yeast-hyphal transition (filamentation) promotes invasion and damage to host tissues. These transitions are associated with different mammalian niches or stages of infection, yet the molecular basis of their preference for these niches is poorly understood. To understand the metabolic differences between phenotypic states, we carried out a comparative analysis of white and opaque cells using Phenotype MicroArrays. This high-throughput analysis allowed monitoring of growth, white-opaque switching and filamentation of *C. albicans* cells under ~2,000 different nutrients or stressors. Integrating the phenotypic data with the annotated genome and the metabolic network of *C. albicans* allowed us to identify metabolic pathways and regulatory processes that are co-regulated with phenotypic transitions. We found that >80% of conditions promoted the growth of white cells to a higher biomass than that of opaques, and that opaque cells were more sensitive than whites to most stressors tested. The increased fitness of white cells was more apparent at 37°C than at 25°C. As expected, switching from white to opaque was favoured by growth at lower temperatures, although several conditions induced this switch at 37°C. We also identified a number of conditions that stabilized the opaque state at 37°C, suggestive of niches where these cells might be stable in the host. Carbon sources were important regulators of the white-opaque switch and subsequent genetic analyses identified glucose sensing and regulatory pathways as key modulators of opaque stability. In parallel, we identified novel nutritional cues that induce the program of filamentation, as well as significant overlaps between the regulation of the white-opaque switch and the yeast-hyphal transition. Our findings underline key differences in the metabolic programs of *C. albicans* cells in alternate phenotypic states. This will allow identification of host niches that promote these phenotypic transitions and understanding of the role of metabolic adaptation in the lifestyle of *C. albicans*..

Fungal bioenergetics drives human fungal pathogenicity: Focus on *Aspergillus fumigatus*. Dawoon Chung, Arsa Thammahong, Sourabh Dhingra, Sarah Beattie, Robert Cramer. Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Over the course of evolutionary history, mammals have evolved elegant mechanisms to resist severe infections by fungi. However, over the last four decades, the incidence of often lethal invasive fungal infections has dramatically risen. While individuals that are susceptible to an invasive fungal infection are most often immune compromised, the spectrum of fungi that causes disease remains limited. Many genes and proteins have been found to be critical for pathogenicity in these select fungi, but it remains to be definitively determined why only certain fungi are capable of causing invasive disease in specific immune compromised individuals. Utilizing the most common filamentous fungal agent of human disease, *Aspergillus fumigatus*, and closely related so called non-pathogens, we are exploring the hypothesis that fungal bioenergetics is the primary driving force behind human fungal pathogenicity. Our hypothesis predicts that measurments of fungal bioenergetics between and within species may allow one to predict the virulence of a given fungal isolate in a given patient. Key genes and biochemical pathways are being identified that drive these virulence associated bioenergetics. Targeting virulence bioenergetics through drug mediated and non-drug mediated mechanisms, in a patient context dependent manner, is a promising novel therapeutic approach to tackle these recalcitrant and devastating infections.

Fumigatin-oxide production by *Aspergillus fumigatus* is regulated by iron availability and temperature involving the transcription factors HapX and SrbA. Beatrix E. Lechner[1], Ernst R. Werner[2], Markus A. Keller[2], Kirstin Scherlach[3], Falk Hillmann[4], Hubertus Haas[1]. 1) Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria; 2) Division of Biological Chemistry, Innsbruck Medical University, Innsbruck, Austria; 3) Department of Biomolecular Chemistry, HKI, Jena, Germany; 4) Department of Molecular and Applied Microbiology, HKI, Jena, Germany.

Iron is an essential metal for the metabolism of virtually all species. For the opportunistic fungal pathogen *Aspergillus fumigatus*, adaptation to iron starvation has been shown to be an essential virulence determinant. Here we found that in *A. fumigatus* liquid cultures, iron starvation induces the secretion of a yellow pigment optimally at 20-25°C, but not at 37°C, within 48-72 h of growth. In contrast, starvation for nitrogen, carbon, phosphate or other metals such as copper or zinc did not trigger production of this extracellular pigment. Deficiency in HapX or SrbA, the master regulators for adaptation to iron starvation and secondary metabolism, respectively, impaired biosynthesis of the pigment. In contrast to *A. fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus* or *Penicillium chrysogenum* were not found to synthesize this pigment. The pigment was purified by solid phase extraction and reversed-phase HPLC separations. High-resolution mass spectrometry revealed a molecular mass of 183.0297 corresponding to the chemical formula of $C_8H_8O_5$. 1H-NMR together

with the photosensitivity and the pH dependence of UV absorption spectra identified the compound as fumigatin-oxide. The aromatic, maroon-colored metabolite fumigatin, showing antibiotic activity against bacteria and toxicity against animals as well as anti-inflammatory activity, was first isolated from *A. fumigatus* culture media in 1938. Subsequently, fumigatin and its derivatives were detected by mass spectrometry in various *A. fumigatus* isolates but the biosynthetic pathway for fumigatin biosynthesis remains to be elucidated. This study represents the first characterization of the regulation of fumigatin production and emphasizes the impact of iron availability on fungal secondary metabolism.

Saturday, March 21 2:00 PM–5:00 PM
Chapel

Effector Biology
Co-chairs: Sophien Kamoun and Regine Kahmann

***Phytophthora sojae* effector Avr1b can be delivered into soybean cells by heterologous PI3P-binding proteins during infection.**
Qunqing Wang, Felipe Arredodo, Eli Perez, Brett Tyler. Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR.

Oomycete and fungal pathogens secrete effector proteins that can enter plant cells to modify the physiology of their hosts. A major class of effectors produced by oomycetes contains RXLR motifs that mediate entry of these effectors into plant cells. We previously showed that RXLR effectors can enter host cells in the absence of any pathogen. Furthermore, these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RXLR motif, plus in some cases, C-terminal regions of the protein. Previously we showed that PI3P binding is required for the effectors to enter into host cells when the purified proteins are introduced into root or leaf tissue. Here we show that the RXLR motif of Avr1b is sufficient for cell entry *in vivo*, independent of the C-terminal PI3P-binding residues. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RXLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicated that Avr1b is post-translationally modified.

The RXLR motif of the *Phytophthora infestans* host targeting effector AVR3a is cleaved before secretion by the pathogen.
Stephan Wawra[1,5], Ian Davidson[2], Uwe Linne[3], Chris J. Secombes[4], Pieter van West[5]. 1) Botanical Institute, Genetical Institute, University of Cologne, Zulpicher Strasse 47b, 50674, Cologne, Germany; 2) Proteomics Facility, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK; 3) Philipps-Universität Marburg, Chemistry, Core Facility for Mass Spectrometry, Hans-Meerwein-Strabe, D-35032 Marburg, Germany; 4) Scottish Fish Immunology Research Centre, Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK; 5) Aberdeen Oomycete Laboratory, College of Life Sciences and Medicine, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.

When oomycete plant pathogens infect their hosts they employ a large arsenal of effector proteins of which some are translocated into their host cells. The RxLR-effectors are the largest group of such molecules defined by their conserved N-terminal Arg-Xaa-Leu-Arg (RxLR) motif. However, the role of this motif in the host-cell translocation process is unclear. We performed detailed biochemical analyses of the RxLR-effector AVR3a from the potato pathogen *Phytophthora infestans* obtained from axenic culture filtrate. High resolution mass spectrometric analyses showed that the RxLR-sequence of AVR3a is cleaved off prior secretion by the pathogen because the mature effector is acetylated at the new N-terminus. These findings suggest a role for the RxLR-motif in the effector export pathway within the oomycete. The observed processing and modification is similar to events observed for Plasmodium export element (PEXEL)-effectors of malaria. Consequently, models of RxLR-mediated pathogen-independent uptake of RxLR-effectors need to be revised.

Effector dynamics during biotrophic invasion by the rice blast fungus, *Magnaporthe oryzae*.
Barbara Valent[1], Mihwa Yi[1,5], Pierre Migeon[1], Ely Oliveira-Garcia[1], Huakun Zheng[1,4], Melinda Dalby[1], Xu Wang[2], Jung-Youn Lee[2], Mark Farman[3], Zonghua Wang[4], Jie Zhou[4]. 1) Dept. of Plant Pathology, Kansas State University, Manhattan, KS; 2) Dept. of Plant and Soil Sciences, University of Delaware, Newark, DE; 3) Dept. of Plant Pathology, University of Kentucky, Lexington, KY; 4) Fujian Agriculture and Forestry University, Fuzhou, 350002, China; 5) Current Address: Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

During biotrophic invasion, *Magnaporthe oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the cytoplasm of rice host cells. The fungus also secretes apoplastic effectors, which remain in the extracellular space between the fungal cell wall and the rice plasma membrane. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocks secretion of apoplastic effectors, but not secretion of cytoplasmic effectors, indicating that *M. oryzae* possesses a distinct Golgi-independent secretory system for targeting cytoplasmic effectors into BICs. Understanding how and where effectors are secreted during biotrophic invasion and how these effectors function to promote blast disease remain major challenges. Biotrophic invasive hyphae secrete many biotrophy-associated-secreted (BAS) proteins, including known effectors. High-throughput in planta localization studies for more than 80 BAS proteins show diverse patterns of temporal regulation of expression and spatial accumulation of secreted proteins. At least 24 BIC-localized effectors are translocated into the cytoplasm of invaded rice cells and move ahead into neighboring rice cells, presumably moving through plasmodesmata to prepare host cells before hyphal invasion. Searching behavior of invasive hyphae before they cross plant cell walls and extreme constriction during crossing led to the hypothesis that these hyphae seek out pit fields containing plasmodesmata for crossing into neighboring cells. We explore potential fungus-plasmodesmata interactions using 7 distinct effectors that accumulate around hyphae as they cross the plant cell wall, and other effectors that undergo cell-to-cell movement.

An assay for entry of pathogen effectors into host cells? L. Lo Presti, R. Kahmann. Organismic interactions, MPI for Terrestrial Microbiology, Marburg, Germany.

A successful colonization of plants by prokaryotic and eukaryotic pathogens requires active effector-mediated suppression of defense responses and host tissue reprogramming. Secreted effector proteins can either display their activity in the apoplast or translocate to host cells and affect plant functions there. While bacterial pathogens use Type III Secretion System (T3SS) for injecting effectors into plant cells the molecular mechanisms of effector delivery by eukaryotic phytopathogens remain elusive. Here we report the establishment of an uptake assay that is based on the ability of a bacterial biotin ligase, BirA, to biotinylate *in vivo* all proteins that carry a small peptide, the Avitag, which serves as substrate for the enzyme. The assay relies on the stable expression of BirA in the cytoplasm of the host plant and the engineering of phytopathogen effectors with the Avitag. The only effectors to be biotinylated by BirA are the ones that translocate into the host cell. Hence biotinylation becomes a hallmark of uptake. We have initially established the feasibility of the assay in a transient expression assay in. *Nicotiana benthamiana*. Currently we are transferring the assay to the *Ustilago maydis*-maize pathosystem and we will present and discuss here our first results.

An RXLR-WY effector of the Irish potato famine pathogen *Phytophthora infestans* antagonizes a host autophagy cargo receptor. Y. Dagdas[1], K. Belhaj[1], A. Maqbool[2], R. Hughes[2], M. J. Banfield[2], S. Kamoun[1], T. O. Bozkurt[1,3]. 1) The Sainsbury Laboratory, The Sainsbury Laboratory, Norwich, United Kingdom; 2) Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK; 3) Imperial College London, Department of Life Sciences, London, UK.

Autophagy is a multifaceted membrane trafficking pathway involved in adaptation to cellular stress conditions such as starvation and pathogen infection. Activation of autophagy leads to formation of special vesicular structures called autophagasomes, which carry autophagic cargo to lysosomes or vacuoles for degradation. A form of autophagy, known as selective autophagy, can specifically degrade particular molecules through autophagy cargo receptors that confine the cargo within a special set of autophagasomes. Although the role of autophagy in antimicrobial defense responses has been documented in animals, the role of autophagy in plant-microbe interactions is unclear and somewhat controversial. Here, we discovered that a secreted RXLR-WY type effector of *Phytophthora infestans*, PexRD54, binds to the autophagy marker protein ATG8. We identified an **A**TG8 **I**nteracting **M**otif (AIM) in PexRD54. Mutations in the AIM prevented both *in vivo* and *in vitro* PexRD54-ATG8 interactions. Consistently, overexpression of PexRD54 increased the number of GFP:ATG8 labeled autophagasomes and enhanced stability of ATG8 protein *in planta*. To investigate the biological function of PexRD54, we studied the autophagy cargo receptor Joka2, which also interacts with ATG8. *In planta* overexpression of Joka2 limited *P. infestans* infection, suggesting a role for Joka2/ATG8 selective autophagy in response to oomycete infection. Remarkably PexRD54, but not the AIM mutant of PexRD54, was able to out-compete Joka2 for binding to ATG8 and restore full pathogen virulence. Our findings point to a model in which a *P. infestans* RXLR-WY effector antagonizes a selective autophagy cargo receptor to enhance pathogen virulence.

Characterization of a *Pyrenophora teres* f. *maculata* mapping population uncovers the complexity of virulence in the spot form net blotch of barley interaction. T.L. Friesen[1,2], S.A. Carlsen[2], J.K. Richards[2], A. Neupane[2], R.S. Brueggeman[2]. 1) Cereal Crops Res Unit, USDA-ARS, Fargo, ND; 2) Department of Plant Pathology, North Dakota State Univ., Fargo, ND USA.

Pyrenophora teres f. *maculata* is a major pathogen of barley worldwide, however, little is known about the virulence underlying this disease. Based on its necrotrophic lifestyle, the pathogen likely produces several necrotrophic effectors (NEs) that elicit NE triggered susceptibility (NETS) in the host. To gain insight into the virulence of *P. teres* f. *maculata*, a mapping population was developed using a cross between *P. teres* f. *maculata* isolates FGOB10ptm (North Dakota) and SG1 (Australia) to derive 105 progeny. The population was phenotyped using nine diverse barley lines including common SFNB differential lines and lines from the world barley core collection put together by the Triticeae Coordinated Agriculture Project (T-CAP). Lines were selected that demonstrated differential reaction to the selected parental isolates. The North Dakota isolate had significantly higher virulence on seven of the barley lines while the Australian isolate had higher virulence on two of the lines. Genotyping was done using a two-enzyme restriction associated DNA (RAD) GBS approach developed for the Ion Torrent PGM. A SNP calling pipeline identified a total of 983 quality SNP markers. These SNP markers were used to develop the first genetic map of *P. teres* f. *maculata*. Co-segregating markers were eliminated leaving 488 informative markers. Markers were distributed across 17 linkage groups generating a total map size of 1780 cM. Using phenotypic and genotypic data, QTL analysis identified more than 20 genomic regions on seven of the linkage groups, associated with *P. teres* f. *maculata* virulence. QTL associated with individual barley lines ranged from three to seven with each line used showing a different QTL pattern. The variation in virulence QTLs between barley lines and the high number of genomic regions associated with virulence indicates a high level of complexity in both pathogen virulence and host resistance or susceptibility.

Effectors from the plant pathogenic *Aphanomyces euteiches* trigger host DNA damage. Elodie Gaulin, Michiel JC Pel, Laurent Camborde, Diana Ramirez, Hélène San-Clemente, Bernard Dumas. UMR5546 CNRS-Université , Laboratoire de Recherche en Sciences Végétales, Castanet-Tolosan, France.

Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes, which are fungal-like eukaryotic microorganisms that cause some of the most destructive plant diseases in the world, secrete several different kind of effector proteins. Two large groups of these effectors are the RXLR and the CRN (Crinkler) proteins. RXLRs and CRNs are modular proteins with conserved N-termini and highly diverse C-terminal effector domains. We recently obtained the genome sequence of the legume root pathogen *Aphanomyces euteiches (ATCC201684, AphanoDBv2.0; https://www.polebio.lrsv.ups-tlse.fr/aphanoDB/)*. This data revealed the absence of RXLR effectors and the presence of over 150 putative CRN effectors in the genome of this pathogen. *Aphanomyces* sp. CRNs are characterized by the presence of an LYLALK translocation motif, and although many CRNs have been identified data on CRN function and targets is still limited. We started the functional analysis of these CRN effectors to gain insights in the virulence mechanisms of *A. euteiches* and to identify possible targets for disease control. We have been able to show that one of the CRN

effectors, CRN13, localizes in the plant nucleus where it triggers cell death. Further, we found that the CRN13 ortholog of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* is able to cause a similar response in both plant and amphibian cells. Additionally, we demonstrated that both CRN13s are able to bind DNA *in vitro* and cause DNA damage *in vivo*. Altogether, this work reveals that CRN effectors produced by unrelated plant and animal pathogens bind DNA to interfere with host cell development.

***F*usarium *R*apid *AL*kalinization *F*actor (*f-ralf*) encodes a secreted virulence effector acquired by horizontal gene transfer from plants.** Sara Masachis[1], David Turrà[1], Mennat El Ghalid[1], Georg Felix[2], Thomas A. Richards[3], Antonio Di Pietro[1]. 1) Department of Genetics, University of Cordoba, Spain; 2) Center of Plant Molecular Biology, Eberhard-Karls-University Tübingen, Germany; 3) Biosciences, University of Exeter, UK.

Fungal pathogens secrete effector molecules to shape the host environment for their own benefit. For instance, extracellular alkalinization is used by a number of phytopathogens to improve colonization of the host tissue. We previously showed that in the root-infecting fungus *Fusarium oxysporum* an increase in extracellular pH promotes invasive growth and pathogenicity on tomato plants. The mechanisms that mediate extracellular alkalinization during fungal infection are poorly understood. Inspection of the *F. oxysporum* genome sequence identified a gene encoding a predicted secreted homolog of *R*apid *AL*kalinization *F*actor (RALF), a conserved family of plant peptide hormones that regulate cell expansion during plant growth and development. Expression of *ralf* is markedly upregulated in *F. oxysporum* during infection of tomato roots. Synthetic F-RALF peptide triggered rapid alkalinization in tomato cell cultures and caused inhibition of root elongation and root hair growth both in tomato and Arabidopsis plants. Fungal mutants lacking the *f-ralf* gene were significantly attenuated in virulence on tomato plants, and induced expression of defense genes in the host. Alkalinization of the extracellular medium was observed in tomato plants exposed to the *F. oxysporum* wild type and complemented strains, but not the *f-ralf* mutants. A survey of sequenced fungal genomes identified *ralf* homologs in a number of phylogenetically distant species, all of them plant pathogens. We propose that these fungi use RALF peptides acquired by horizontal gene transfer from plants to enhance their infectious potential through alkalinization of the host tissue.

Saturday, March 21 2:00 PM–5:00 PM
Fred Farr Forum

Dynamics of Genome Evolution
Co-chairs: Daniel Croll and Li-Jun Ma

Evolutionary constrains of host specificity in the smut fungus *Microbotryum*. Britta Bueker[1,2], Michael Hood[2], Dominik Begerow[1]. 1) Ruhr-Universität Bochum, AG Geobotanik, Universitaetsstr. 150, 44780 Bochum, Germany; 2) Amherst College, Biology Department, 01002 Amherst, MA, USA.

In fungal pathogens, the occurrence of interspecific hybridization is often linked to host species´ distribution and characteristics. Therefore, analysis of the genetics underlying host specialization is a crucial factor for understanding pathogen´s evolution and the involved mechanisms. In the current study we use the basidiomycetes smut fungus *Microbotryum* – a species complex with independent evolutionary lineages that typically specialize to a given host plant species – to study genetic determinants of hybridization and host specialization. To do so, we are analyzing and comparing the genomes of the two *Microbotryum* species, *M. lychnidis-dioiceae* and *M. silenes-acaulis* – both species that are well adapted to distinct host environments. The use of hybrids and selective infection experiments allows us to assess genomic constraints on hybrid viability and to isolate the effect of the mating type chromosomes in relation to host adaptation. The results suggest that loci involved in the disease interactions may be associated with the mating type chromosomes. Furthermore, by focusing on the occurrence of genes underlying positive selection in F1 hybrids and selected backcrosses, potential candidate genes that play a crucial role in infection and virulence can be described. Thus, the application of selective infection experiments in combination with genomic analysis is a feasible approach to elucidate the evolutionary forces of host specificity in the *Microbotryum* pathogen complex..

Transposable elements reshaping genomes and favoring the evolutionary and adaptive potential of fungal phytopathogens. Thierry Rouxel, Jonathan Grandaubert, Marie-Helene Balesdent. INRA-Bioger, Thiverval-Grignon, France.

Transposable Elements (TEs) have been considered for long as “junk” DNA in the genome of complex eukaryotes. However, massive sequencing efforts coupled with phylogenetic analyses suggest TEs can act as genome shapers and be a source of gene innovation and genome plasticity, eventually contributing to genome divergence. Fungi are simple and easy to manipulate eukaryote organisms, for which the ever-growing genome information indicates that many plant-associated fungi have a tendency towards genome size expansion. This increase in genome size is mostly driven by TE expansion that eventually shapes adaptive regions of the genome. Such genome regions host genes involved in niche adaptation and favor accelerated evolutionary dynamics of these genes. Focusing on the *Leptosphaeria maculans-Leptosphaeria biglobosa* species complex of closely related plant pathogenic fungi, we will discuss the link between TE invasion/TE bursts in genomes and (i) speciation, (ii) the rise of two-speed genomes, shaping plastic genome environments, (iii) gene diversification that contributed to adaptation to new hosts, (iv) heterochromatine-based regulation of expression of effector genes, (v) accelerated adaptation to resistance gene pressure in gene-for-gene systems.

Genome plasticity mediated by transposable elements drives the evolution of virulence in the vascular wilt pathogen *Verticillium dahliae*. Michael Seidl, Luigi Faino, David Cook, Xiaoqian Shi-Kunne, Grardy van den Berg, Bart Thomma. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Verticillium dahliae is a soil-borne pathogen that aggressively colonizes hundreds of host plants, including high value crops such as tomato and potato, leading to the formation of vascular wilt disease. Resistance in the host population exert selective pressure on the pathogen forcing the rapid evolution of adaptive traits to successfully participate in the arms race with the host. By comparative genomics of the *V. dahliae* population, we recently revealed genomic rearrangements that facilitate the gain and loss of genetic material and establish highly dynamic lineage-specific (LS) regions. LS regions are enriched for transposable elements (TEs) and *in planta* induced effector genes encoding secreted protein that significantly contribute to aggressiveness towards the host, and thus have been hypothesized to contribute to the genome plasticity required for adaptive genome evolution. However, the factors that drive genome plasticity in *V. dahliae* remain enigmatic. Using long-read sequencing technologies, we re-sequenced two *V. dahliae* strains and analyzed the previously identified genomic rearrangements in unprecedented detail, revealing multiple genomic breakpoints down to the nucleotide level. Genomic breakpoints are flanked by multiple TEs, suggesting that these elements play essential roles in their formation. Comparative analyses of *V. dahliae* with the recently sequenced non-pathogenic *Verticillium tricorpus* revealed a highly expanded TE repertoire in pathogenic *V. dahliae*, where *in planta* induced effector candidates, but also other genes encoding secreted proteins, are frequently flanked by TEs. Additionally, whole-genome bisulfite sequencing of *V. dahliae* identified DNA methylation predominantly targeting TEs. In fungi, inactivation of TEs by DNA methylation is common, and we hypothesize that it could also influence the expression of nested effector candidates, thereby providing yet another route how TEs can affect the interaction between the pathogen and its host. In summary, we highlight the profound role of TEs on the evolution of virulence in the vascular wilt pathogen *V. dahliae*.

The origin and fate of chromosomal structural variants in fungal populations. Daniel Croll. ETH Zurich, Zurich, Switzerland.

Fungal pathogen populations show extraordinary potential to adapt to changes in the environment, host genotypes or chemical control agents. The main determinants of rapid evolution are likely to be extensive standing genetic variation, frequent sexual reproduction and high dispersal capabilities. Despite the ubiquity of evidence for rapid turnover occurring in fungal populations, little is known how the structure of the genome influences the evolution of genetic variation. Most studies are focused on allele frequency changes at SNP loci. However, chromosomal rearrangements and variations in recombination rates are expected to significantly influence the evolutionary potential of loci. We aimed to comprehensively study chromosomal structural variants segregating in fungal populations of the ascomycete wheat pathogen *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*). First, we generated near-complete genome assemblies of four field isolates using a combination of short- (Illumina) and long-read (PacBio) technologies, and high-resolution genetic maps. Comparative analyses of the genome assemblies showed that a substantial proportion of the polymorphism in a field population is due to structural variation in the chromosomal sequences. Second, we used re-sequencing data from 130 additional isolates from a global collection to identify the frequency of segregating structural variants. Third, we calculated genome-wide linkage disequilibria and correlated the level of linkage disequilibria with the presence of structural variation. We found that population linkage disequilibria were higher near chromosomal rearrangements breakpoints. The chromosomal location of structural variants also correlated with variations in recombination rates. The presence of segregating structural variants within populations is likely to affect the genetic architecture of phenotypic traits and provides an important genomic context to predict the evolutionary potential of loci in a pathogen genome.

Tracing the evolutionary trajectory of drug resistance and virulence in clinical isolates of *Candida albicans* . Chris B. Ford[1], Jason M. Funt[1], Darren Abbey[3], Luca Issi[4], Candace Guiducci[1], Toni Delorey[1,4], Theodore C. White[5], Reeta P. Rao[4], Judith Berman[3,6], Aviv Regev[1,2], Dawn Thompson[1]. 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 Massachusetts Ave, Cambridge, MA 02140; 3) University of Minnesota, Minneapolis MN 55455 USA; 4) Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester MA 01609; 5) School of Biological Sciences, University of Missouri at Kansas City, MS; 6) Tel Aviv University, Ramat Aviv, 69978 Israel.

Candida albicans is both a member of the healthy human microbiome and a major pathogen in immune-compromised individuals. Infections are typically treated with azole inhibitors of ergosterol biosynthesis that often leads to drug resistance. Since *C. albicans* is a predominately asexual diploid, conventional genetic analysis is challenging. Another approach is to study the mutations arising naturally during the evolution of drug resistance *in vivo*, using isolates sampled consecutively from the same patient. We leveraged next-generation sequencing to analyze 43 isolates from 11 oral candidiasis patients. We detected newly selected mutations, including single-nucleotide polymorphisms (SNPs), copy-number variations and loss of heterozygosity (LOH) events. LOH events were commonly associated with acquired resistance, and SNPs in > 150 genes may be related to host adaptation. Conversely, most aneuploidies were transient and did not correlate with drug resistance. Our work reveals new molecular mechanisms underlying the evolution of drug resistance and host adaptation. Finally, we are investigating the molecular underpinnings of host adaption in these isolates with our newly developed host pathogen RNA seq pipeline.

Genome dissection of a *Fusarium oxysporum* isolate that causes fusariosis. Yong Zhang, Greg DeIulio, Li Guo, Li-Jun Ma. biochemistry & molecular biology, UMass, Amherst, Amherst, MA.

Advances in medical treatments increase the complexity of patient populations with immunodeficiency disorders. Opportunistic fungi have emerged as important causes of morbidity and mortality in immunocompromised individuals. Fusariosis, the infection caused by *Fusarium* spp., is the second most common opportunistic infection caused by filamentous fungi, after aspergillosis. *F. oxysporum* causes localized or disseminated infections that may become life-threatening in neutropenic individuals. Some members of the *F. oxysporum* species complex also cause devastating plant wilt diseases. Horizontally transferred supernumerary (SP) chromosomes determine host-

specific pathogenicity of plant-infecting *F. oxysporum* isolates; however, it is unknown whether SP chromosomes also contribute to the increasing cases of fusariosis in humans. Here we dissected the genome of the clinical *F. oxysporum* isolate NRRL32931. Optical mapping technology revealed a compartmentalized genome structure with four unique SP chromosomes (1.8, 1.3, 1.2, and 1 Mb in size). Functional annotation of the 812 genes encoded by the SP chromosomes indicates a potential role in survival and proliferation of the pathogen under high temperature, high pH and ion-poor conditions, similar to those encountered in the human body. This study provides the first evidence for the presence of SP chromosomes in a human-infecting fungus, revealing a potential role of genome compartmentalization in rapid adaptation of *F. oxysporum* to the human host and in the establishment of invasive mycoses.

Sex determination in the red yeast *Rhodosporidium babjevae*: exploring the impact of genomic structural variation. Marco A. Coelho, Susana Lopes, José P. Sampaio, Paula Gonçalves. Centro de Recursos Microbiológicos (CREM), Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

Sexual reproduction is ubiquitous and extant in all of the major groups of the eukaryotic tree of life. In the fungal kingdom, the increasing number of completed genome sequences has shown that the evolution of reproductive systems is a highly dynamic process.

Within basidiomycetes, many yeast species have developed a unique tetrapolar mating system determined by two unlinked genomic regions: (i) a pheromone/receptor (P/R) locus that is often biallelic and coordinates cell recognition/fusion; and (ii) a multiallelic homeodomain (HD) locus that regulates the progression through the sexual cycle. Since alleles at both loci must differ for sexual reproduction to occur, tetrapolar species seem to efficiently restrict inbreeding (25%), while potentially supporting efficient outcrossing (≥50%). The molecular basis of the tetrapolar mating system has been so far characterized with detail in species of the Ustilaginomycotina and Agaricomycotina subphyla. Using phylogenetic, comparative genomics and electrophoretic karyotype analyses, we now provide a detailed description of the tetrapolar sexual system in the red yeast *Rhodosporidium babjevae* therefore expanding the characterization of this system to the earliest derived lineage of the Basidiomycota, the Pucciniomycotina subphylum. Besides defining the genetic structure of *P/R* and *HD* loci in compatible strains of *R. babjevae* using whole-genome sequencing and chromoblot analyses, a collection of 21 additional wild strains of *R. babjevae* revealed the presence of 16 *HD* alleles and only two *P/R* alleles. Recombination between both *MAT* loci seems to be allowed as assessed in the progeny of a cross between *R. babjevae* compatible strains, but most of the progeny had reduced (sexual) reproductive fitness, particularly those with assorted *P/R* and *HD* loci. We are currently exploring the impact of intraspecific genomic structural variation in both sex determination and reproductive isolation.

Unisexual Reproduction - Climbing the Hill, Robertson Effect, without a Partner. Kevin Roach, Joseph Heitman. Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans is a pathogenic basidiomycetous fungus that >1,000,000 infections and 600,000 deaths annually. Despite the presence and comparable fitness of both mating-types, α isolates predominate (>95 - 99%) in both nature and patients. Long thought to be asexual in many environmental, *C. neoformans* undergoes opposite sex mating (**a**-α) under lab conditions and was recently discovered to undergo a novel form of unisexual mating (α-α). The ability to sexually reproduce without an opposite mating-type partner raises questions about the evolutionary impact of unisexual reproduction on predominantly *MAT*α populations. One such question is the effect of unisexual reproduction on the Hill-Robertson effect, a reduction in effectiveness of selection due to interference in selection between linked loci. In asexually dividing populations a beneficial mutation that occurs on a background with deleterious mutations may be lost due to purifying selection on the background. Likewise, two advantageous mutations segregating on different backgrounds will compete and rise in frequency more slowly unless recombination brings them together on the same background. If *C. neoformans* populations, were limited to only rare opposite mating-type sexual reproduction because of the preponderance of α mating-type, selection would have less opportunity to act on individual alleles and would instead act on whole genotypes. We tested whether unisexual reproduction between *MAT*α individuals reduces linkage disequilibrium between mutations. We show that unisexual reproduction can break linkage between advantageous and deleterious mutations, as well as bring advantageous mutations into linkage together on the same background, increasing the effectiveness of selection. Unisexual reproduction therefor reduces linkage and allows selection to act on individual alleles to reduce the Hill-Robertson effect, thereby increasing the fitness of populations of finite size.

Saturday, March 21 2:00 PM–5:00 PM
Kiln

Extremophilic Fungi
Co-chairs: Ana Plemenitas and Igor Grigoriev

Thermophilic fungi: phylogeny, genomics, and degradation of plant-derived biomass. Adrian Tsang[1,2], Ian Reid[2], Nicholas O'Toole[2], Annie Bellemare[2], Ingo Morgenstern[1,2], Nadeeza Ishmael[2], Erin McDonnell[2], Kimchi Strasser[1,2], Carol Nyaga[2], Gregory Butler[2], Justin Powlowski[2]. 1) Biol, Concordia Univ, Montreal, Canada; 2) Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada.

Filamentous fungi are the major decomposers of plant-derived biomass. They secrete a wide array of enzymes to hydrolyze polysaccharides (cellulose, hemicellulose, pectin) and to mineralize lignin, the major polymers found in plant cell wall. Thermophilic fungi are of particular interest because they are potential reservoirs of thermostable enzymes for industrial applications. Thermophily in fungi and the taxonomic placement of many thermophilic fungi are not well established. Adopting the definition for fungal thermophily as fungi with

optimal temperature of growth of above 40-45°C, about 30 reported species can be classified as thermophilic fungi. Most of these thermophiles belong to the orders Sordariales and Eurotiales, with three species belong to the Mucorales and one to Onygenales. We have sequenced the genomes of these thermophiles, many to very high quality. Thermophilic fungi are currently the only filamentous fungi with finished, gapless genomes. We have completed the manual curation of all the genes in the finished genome of *Remersonia thermophila.* The genome sequences have helped to correct the taxonomic placement of previously misplaced thermophilic species. An effort facilitated by the Joint Genome Institute is underway to sequence mesophilic and thermotolerant species that are related to the thermophiles to elucidate the evolution of fungal thermophily. The genome resource for the thermophilic fungi can be accessed via www.fungalgenomics.ca.

Over 4000 genes encoding potential lignocellulolytic proteins have been identified from the fungal thermophiles. 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 shows that most of the enzymes originated from the fungal thermophiles have temperature optimum in the 50-70°C range.

Thermophilic fungi for efficient plant biomass degradation. Joost van den Brink, Benjamin Stielow, Ronald de Vries. CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.

A major challenge in the sustainable production of biofuels and biochemicals is efficient enzymatic conversion of plant biomass into monomeric sugars. Most enzyme mixtures are currently produced by a small selection of fungal species (e.g. *Trichoderma reesei*, *Aspergillus niger*). However, the fungal kingdom holds many more fungal species which produce enzyme mixtures with beneficial characteristics such as high (hemi-)cellulase activity and high thermostability. *Sordariales* is one of the few fungal orders with thermophilic isolates, of which many have been associated with the production of thermostable enzymes. The goal of our study is to assess the diversity within *Sordariales* for efficient plant biomass degradation. *Sordariales* isolates showed a large variety of optimal growth temperatures ranging from 25°C till 45°C. Phylogenetic analysis, using a novel highly multiplexed targeted next generation sequencing approach, revealed that optimal growth temperature is a polyphyletic trait within *Sordariales* with separate mesophilic, thermotolerant and thermophilic clades. Four thermophilic clades were clearly distinguished: *Myceliophthora* species, *Thielavia terrestris*, *Chaetomium thermophilum*, and *Mycothermus thermophilus*. Interestingly, enzyme characteristics followed the optimal growth temperatures and were a strong polyphyletic trait. Thermophiles within *Myceliophthora* showed the most potential as efficient plant biomass degraders. Especially *M. heterothallica* had good growth on a large range of substrate and was able to produce offspring with a large physiological and genetic variety. Mating and evolutionary engineering strategies were used to further improve *M. heterothallica* capability to degrade biomasses such as sugarbeet pulp and spruce. In conclusion, this study showed the strategic strength of combining fungal diversity with specific selection strategies to find enzyme mixtures with interesting industrial properties.

Experimental and genomic approaches to understanding the reproductive systems of the thermophile Myceliophthora heterothallica and other members of the Chaetomiaceae. M. I. Hutchinson[1], A. J. Powell[2], A. Tsang[3], R. M. Berka[4], N. O'Toole[3], I. V. Grigoriev[5], K. Barry[5], A. Robinson[1], D. O. Natvig[1]. 1) Department of Biology, University of New Mexico, Albuquerque, NM, USA; 2) Sandia National Laboratories, Albuquerque, NM, USA; 3) Concordia University, Centre for Structural and Functional Genomics, Montreal, QC, CA; 4) Novozymes Inc., Davis, CA, USA; 5) DOE Joint Genome Institute, Walnut Creek, CA, USA.

Members of the Chaetomiaceae are among the most reported species in studies of biomass degradation. This family also contains many of the best-known thermophilic fungi, which are of industrial interest for their abilities to produce thermostable carbohydrate active enzymes. Since there has been no genetically tractable model for the Chaetomiaceae, we have characterized sexual reproduction in the thermophile *Myceliophthora heterothallica* with the goal of establishing this organism as a model for the group. Genome sequences from *M. heterothallica* as well as other members of the Chaetomiaceae show that protein-coding genes in the mating regions of heterothallic species are conserved relative to other outcrossing Sordariales, with an important exception: mating-type *a* strains from heterothallic species of Chaetomiaceae possess a partial *mat A-1* gene. The *mat A-1* in mating-type *a* strains is truncated for the alpha box region required for fertility in other Sordariales, although it has a conserved intact downstream reading frame. Among species of Chaetomiaceae with teleomorphic states, heterothallism is rarely reported while homothallism is common. Analysis of genomes of homothallic species demonstrates that they possess a true *mat a-1* gene, but it is not linked to the *mat A-1* region. Among filamentous Ascomycota, this represents one recurring evolutionary pathway for derived homothallism, which requires *mat A* and *mat a* regions in a single haploid genome. Phylogenetic analyses reveal that temperature growth responses are evolutionarily plastic within the family, with some clades possessing both thermophiles and mesophiles. These analyses also highlight taxonomic problems, including paraphyly in several groups.

Preliminary characterization of biofuel relevant phenotypes in a natural population of *Kluyveromyces marxianus* for genome wide association study. Marie K. Donnelly[1,2], Jasmine Yu[2], Jacob Baker[2], Thomas D. Bruns[1,2], John W. Taylor[1,2]. 1) Plant and Microbial Biology, University of California Berkeley, Berkeley, CA; 2) Energy Biosciences Institute, University of California Berkeley, Berkeley, CA.

A chief technical hurdle to industrial cellulosic biofuel production is *Saccharomyces cerevisiae's* sensitivity to high temperatures and toxic products of biomass pretreatment and hydrolysis in the fermentation. While much work has been done to engineer suitable industrial strains, a complementary approach is to look for desirable traits in closely related yeasts, such as the thermotolerant yeast, *Kluyveromyces marxianus*. Few strains of *K. marxianus* exist in culture collections, and the extent of natural variation in traits like thermo-tolerance and pH sensitivity is unknown. To sample the natural variation of *K. marxianus*, we traveled to the sugarcane growing and refining region of Florida and collected sugarcane bagasse from active compost piles where the temperature was between 40 and 50°C. 120 bulk bagasse samples were collected from four geographically distant sites. Bagasse samples were blended with water to create slurries, diluted by 10X

and 100X, plated on YM agar at neutral pH and acidic pH conditions, and incubated at 45°C until cultures grew. Cultures were identified by BLAST matches of the amplified ITS region. Cultures identified as *K. marxianus* were isolated and grown up in liquid media to assess phenotypic variation within this population. Genomic DNA was extracted from each individual and sequenced via Illumina HiSeq. Preliminary results show variation within the population in growth above 45°C. In addition to thermo-tolerance, we will investigate variation in response to inhibitors in the growth medium, anaerobic tolerance to ethanol, and utilization of carbon. The goal of this work is to match the phenotypic variation in this natural population with genetic variation using the technique of genome wide association.

Genome sequencing of four *Aureobasidium pullulans* varieties unravelled their stress tolerance, biotechnological potential, pathogenicity and enabled description of four new species. Nina Gunde-Cimerman[1,2], Robin A. Ohm[3], Tina Kogej[1], Silva Sonjak[1], Martina Turk[1], Janja Zajc[1], Polona Zalar[1], Martin Grube[4], Hui Sun[3], James Han[3], Aditi Sharma[3], Jennifer Chiniquy[3], Chew Yee Ngan[3], Anna Lipzen[3], Kerrie Barry[3], Igor V. Grigoriev[3], Cene Gostinčar[1,5]. 1) Biology, Biotechnical Faculty, University of Ljubljana, BF, Ljubljana, Slovenia; 2) 2Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Jamova 39, SI-1000 Ljubljana, Slovenia; 3) US Department of Energy Joint Genome Institute, 2800 Michell Drive, Walnut Creek, CA 94598, USA; 4) Institute of Plant Sciences, Karl-Franzens-University Graz, Holteigasse 6, A-8010 Graz, Austria; 5) National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia.

Aureobasidium pullulans is a black-yeast-like fungus used for production of the polysaccharide pullulan, the antimycotic aureobasidin A, and as a biocontrol agent in agriculture. It can cause opportunistic human infections, and it inhabits various extreme environments. To promote the understanding of these traits, *de-novo* genome sequencing of the four varieties of *A. pullulans* was performed. The 25.43-29.62 Mb genomes of these four varieties of *A. pullulans* encode between 10266 and 11866 predicted proteins. Their genomes encode most of the enzyme families involved in degradation of plant material and many sugar transporters. They have genes possibly associated with degradation of plastic and aromatic compounds, synthesis of pullulan and siderophores. Putative stress-tolerance genes include several aquaporins and aquaglyceroporins, large numbers of alkali-metal cation transporters, genes for the synthesis of compatible solutes and melanin, all of the components of the high-osmolarity glycerol pathway, and bacteriorhodopsin-like proteins. All of these genomes contain a homothallic mating-type locus. The differences between these four varieties are large enough to justify their redefinition as separate species: *A. pullulans, A. melanogenum, A. subglaciale* and *A. namibiae*. Available genome sequences should improve their biotechnological exploitation and promote our understanding of their diverse lifestyles, stress-tolerance mechanisms and pathogenic potential.

Stress resistance of black yeasts and microcolinal fungi: a matter of fine tuning on the proteome and transcriptome level. K. Sterflinger[1], D. Tesei[1], B. Blasi[1], G. Marzban[1], M. Marchetti-Deschmann[2], H. Tafer[1]. 1) BOKU Vienna, Extremophile Center, Vienna, Austria; 2) Technical University Vienna, Institute of Chemical Technologies and Analytics.

Black yeasts and black microcolonial fungi today are assumed to be the most stress resistant Eukaryotes known on Earth. They are typical inhabitants of extreme environments as rock in hot and cold deserts, glaciers, rocky shores, salterns, acidic and alkaline environments but occur also in niches of the human environment. Black fungi are oligotrophic and tolerate extreme temperature ranges, desiccation, high UV- and radioactive radiation as well as osmotic and oxidative stress. Two model organisms are currently within the focus of research on stress resistance in fungi: (1) Cryomyces antarcticus, an extremophilic fungus from Antarctic dry valleys with narrow ecological amplitude and (2) Exophilala dermatitidis, a poly-extremophilic fungus with wide ecological amplitude and natural reservoirs in glaciers and tropical regions. E. dermatitidis is also the causative agent of various severe illnesses in humans. In this study we present results of proteomic analyses under varying culture conditions as well as the functional analysis of the transcriptional response of E. dermatitidis. Interestingly, a major part of the proteome of C. antarcticus has a low identification rate based on sequence homology and thus varies significantly from other fungal species. Incubation conditions did not affect the protein pattern dramatically in E. dermatitidis, thus supporting the strain`s adaptations to extreme conditions and suggesting the presence of a set of proteins relevant to tolerance. By transcriptomic studies we further demonstrate that apart from the protein coding genes, non-coding RNAs, circular RNAs as well as fusion-transcripts are differentially regulated and that the function of the fusion-transcripts can be related to the corresponding temperature condition. This work establishes that E. dermatitidis adapts to its environment by modulating coding and non-coding gene transcription levels and through the regulation of chimeric and circular RNAs. The findings are a first step towards understanding the transmission and virulence of the emerging pathogen E. dermatitidis.

Rock-weathering fungi: a simple genetically tractable model system to study organism-material interactions. Anna A. Gorbushina[1,2], Nicole Knabe[1]. 1) Materials and Environment, Federal Inst. of Materials Research and Testing, Berlin, Germany; 2) Freie Universität Berlin, Berlin, Germany.

So-called rock-inhabiting microcolonial ascomycetes colonise living and inert atmosphere-exposed surfaces, representing the relevant model for material biodeterioration studies. The fungal growth habit contributes significantly to close contact with the colonised substrate and accompanying microorganisms. Microcolonial fungi (MCF) from rock biofilms are responsible for substrate penetration, as well as for the synthesis of extracellular polymeric substances and stimulation of interorganism contacts.

Multigene phylogenetic studies showed that rock-inhabitant lifestyle evolved in two classes of ascomycetes, namely Dothideomycetes and Eurotiomycete. An appropriate representative of MCF to study stress tolerance and evolutionary issues is *Knufia petricola* strain A95 (= CBS 123872), which was isolated from a marble rock surface in Athens (Greece). As the rock-inhabiting *K. petricola* A95 belongs to an ancestral lineage of the Chaetothyriales and is a predecessor of opportunistic pathogens and lichens, it is a well-suited model to study organism-material interactions as well as of potential importance for understanding the evolution of ascomycetous life styles. *K. petricola* A95 is easy to handle, physiologically characterised and genetically tractable. Recently, The Black Yeast Project of the Broad Institute

(Boston, MA, USA) released the annotated genome of A95 and related fungi. In collaboration with different laboratories we are using comparative genome sequence analysis as well as genetic methods to investigate the mechanisms of the remarkable stress tolerance and material colonisation behaviour of A95. Further, the recent establishment of a DNA-transfer- and integration system into *K. petricola* A95 allows for construction of pigment knock-out mutants and thus sets the stage for a variety of investigations into the role of rock-inhabiting fungi in biofilm-induced material deterioration.

***Exophiala dermatitidis* as a model for investigating the stress biology of extremophile fungi.** Jyothi Kumar, Erin Creasey, Steven D Harris. Center for Plant Science Innovation, University of Nebraska Lincoln, NE.

Extremophiles are broadly recognized as organisms that can live in extreme conditions of temperature, acidity, alkalinity, or salinity. Besides expanding our views on the diversity of life on Earth and perhaps beyond, the study of extremophiles has also provided significant insight into how organisms adapt to stress. Extremophile fungi that primarily colonize exposed rock surfaces, known as "rock inhabiting fungi' (RIFs), were originally discovered in Antarctica, but have since been found throughout temperate habitats. RIFs possess numerous morphological and physiological traits and their oligotrophic lifestyles allow them to thrive in harsh environments that are otherwise detrimental. We propose that the "model" black yeast *Exophiala dermatitidis* can be used to investigate the molecular basis of these traits. The accumulation of pigments, melanin and carotenoids likely play a key role in the stress tolerance of *E. dermatitidis.* In their functional characterization of the GTPases Cdc42 and Rac1, Guo and Szaniszlo (unpublished results) noted that the absence of Cdc42 resulted in apparent loss of carotenoids. We have confirmed this and further shown that Cdc42 mutants fail to induce the carotenoid gene cluster. We are currently using transcriptome sequencing to better define the role of Cdc42 in pigment synthesis and broader stress responses. We have also discovered that *E. dermatitidis* can engage in transient mutualisms with photosynthetic algae to support growth and development. This was accomplished by co-culturing *E. dermatitidis* with the alga *Chlorella sorokiniana* in the presence of light on a minimal medium that otherwise lacked carbon. Preliminary results show that co-cultivation markedly enhances growth, promotes the formation of hyphae, triggers asexual development, and results in stable associations between fungal and algal cells. These results suggest that transient mutualisms may play a significant role in enabling the survival of extremophile fungi in harsh environments.

Saturday, March 21 2:00 PM–5:00 PM
Heather

ROS in development and pathogenicity
Co-chairs: Paul Tudzynski and Philippe Silar

The tetraspanin PLS-1 is required for NOX-1 and NOX-2 functions in cell fusion, cell growth and differentiation in the fungus *Neurospora crassa*. M. Hernández-Galván[1], N. Cano-Domínguez[1], A. Robledo-Briones[2], E. Castro-Longoria[2], A. Lichius[3], J. Aguirre[1]. 1) Departamento de Biología Celular y Desarrollo. Instituto de Fisiología Celular UNAM. Apartado Postal 70-242 México City, D. F., México; 2) Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), 22860 Ensenada, B.C. México; 3) Institute of Chemical Engineering, Research Division Biotechnology and Microbiology, University of Technology, 1060 Vienna, Austria.

The NADPH oxidases (NOX) are enzymes that catalyze the regulated production of superoxide. *Neurospora crassa* contains NADPH oxidases NOX-1 and NOX-2. NOX-2 is essential for ascospore germination; while NOX-1 is required for sexual and asexual development, polar growth and the cell fusion that occurs through conidial anastomosis tubes or CAT, during asexual spore germination. A common regulatory subunit NOR-1 is essential for these NOX functions. Tagging of NOX-1 and NOR-1 with GFP to determine their subcellular localization shows a fluorescence signal consistent with perinuclear ER and vacuole localization during CAT fusion, while in growing hyphae it was found in punctuated and cable-like patterns, and notably enriched in septa and plasma membrane at the hyphal tips. During hyphal growth NOX-1::GFP and NOR-1::GFP showed similar but not identical localization, suggesting the specifics sites at which NOX-1 is active. Tetraspanins are small integral membrane proteins that act as organizers of membrane signalling complexes. So far three tetraspanin families (PLS-1, TSP-2 and TSP-3) have been identified in fungi and PLS-1 members have been specifically related to NOX-2 function. We generated Δ*pls*-1 mutants in *N. crassa* and show that these mutants display phenotypes that are identical to those found in *nox-1* and *nox-2* mutants, indicating that in this fungus PLS-1 is required for NOX-1 and NOX-2 function. Tagging of PLS-1 with mCherry at its N-terminus results in a protein that is partially functional and, like NOX-1, is enriched in septa and hyphal tips. Supported by grants CB 153256 from CONACYT, IN207913 from PAPIIT-UNAM and DFG-CONACYT Germany-Mexico collaboration grant 75306.

Interplay of MAPK and ROS signaling in chemotrophic growth of *Fusarium oxysporum*. Daniela Dirschnabel, David Turrà, Antonio Di Pietro. Department of Genetics, University of Córdoba, Córdoba, Spain.

Chemotropism, the ability to re-orient the growth axis in response to chemical cues, is critical for many aspects of fungal lifestyle such as colony establishment, foraging for nutrients or location of host organisms. We use the root-infecting pathogen *Fusarium oxysporum* as a model to study various aspects of chemotropic signaling such as the perception of chemoattractants from the host plant. Previous studies revealed that chemotropism towards nutrients, sex pheromones or plant signals is governed by distinct mitogen-activated protein kinase (MAPK) cascades. The major chemoattractant secreted by tomato roots was identified as a peroxidase, an enzyme that catalyzes the reductive cleavage of H_2O_2. Together with the observation that chemoattraction towards tomato roots is abolished by the antioxidant ascorbate, this finding points towards a role of reactive oxygen species (ROS) in activation of the chemotropic response. The ROS-generating enzymes NADPH oxidases (Nox) were previously shown to regulate developmental processes requiring chemotropic growth such as hyphal fusion and plant infection. We have created deletion mutants in the NADPH oxidases NoxA or NoxB, as well as in the

associated regulator NoxR. The phenotypes of these mutants are currently being characterized in order to determine the impact of NOX in MAPK-mediated chemotropic growth of *F. oxysporum.*.

Two closely related Rho GTPases, Cdc42 and RacA, have opposite roles for the ROS production and symbiotic infection of endophytic fungus *Epichloë festucae*. Daigo Takemoto, Yuka Kayano. Graduate Sch Bioagricult, Nagoya Univ, Nagoya, Japan.

Epichloë festucae is an endophytic fungus systemically colonizes the intercellular spaces of temperate grasses to establish symbiotic associations. Hyphae of the endophyte grow by tip growth in culture and meristematic tissue of host plant, whereas they divide and extend by intercalary growth in the host leaf tissues to maintain the synchronized hyphal growth with leaf expansion of the host plant. We have shown that ROS produced by a specific NADPH oxidase isoform, NoxA, and its regulators, NoxR and small GTPase RacA, have a critical role in regulating hyphal growth in the host plant. BemA and Cdc24, homologues of polarity establishment proteins of yeast, were also identified as interactors of NoxR. In this study, functions of RacA and closely related small GTPase Cdc42 were characterized. L-012-mediated detection of ROS production at the edge of hyphal colonies indicated that RacA and Cdc42 positively and negatively regulate the ROS production. In host plant, *racA* mutant showed irregular blanching and growth, which induce stunting and premature senescence of the host. In contrast, growth of host was not affected by the infection of *cdc42* mutant. *cdc42* mutant can colonize meristematic tissue of the host plant as wild type, however, fragmentation of hypha was observed at the bottom part of host plant, and no hypha was detected at the top of leaf blade, indicated that Cdc42 is required for the intercalary extension of endophyte hyphae in host leaf tissues. Yeast two hybrid assay indicated that RacA and Cdc42 specifically interact with NoxR and BemA, respectively. By domain swap and point mutations, amino acid residues for their specific binding were identified. Introduction of mutated RacA, which lost binding activity to NoxR, can recover the ROS production in culture, colony morphology and polarized hyphal growth of *racA* mutant, but hyphal fusion and symbiotic infection was not complemented. NoxR-binding form of Cdc42 can complement the hyphal fusion and symbiotic infection of *racA* mutant, but ROS production and morphological disorders were not recovered. These results highlight the multifunctionality of these small GTPases and importance of binding specificity for their functional differentiation.

Investigating the role of NADPH oxidases during infection related development of the rice blast fungus *Magnaporthe oryzae*. Lauren Ryder, Kenichi Ikeda, Magdelena Martin-Urdiroz, Michael Kershaw, Nicholas Talbot. Dept Biosciences, Univ Exeter, Exeter, United Kingdom.

Rice blast disease is a major threat to global food security and remains difficult to control. The rice blast fungus *Magnaporthe oryzae* infects plants with a specialized single-celled infection structure called an appressorium, which generates high internal turgor to drive a rigid penetration peg through the rice leaf cuticle.. 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 signaling, and apoptosis, whereas in plants Nox are necessary for programmed cell death, the response to environmental stresses, pathogen infection, and polarized 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-polarized to polarized cell growth, such as tissue invasion by mutualistic and pathogenic fungi, and fungal virulence. We have shown that Nox are essential for septin-mediated re-orientation of the F-actin cytoskeleton to facilitate cuticle rupture and plant cell invasion. Moreover, the Nox2-NoxR complex, in particular, is essential for spatial organization of a hetero-oligomeric septin ring at the appressorium pore, necessary for assembly of a toroidal F-actin network at the point of penetration peg emergence. We have also demonstrated a direct effect of reactive oxygen species on F-actin polymerisation and appressorium function. We have investigated the downstream transcriptional consequences of the loss of Nox2 function, by global transcriptional profiling, and have also determined the physical interactions that occur via the Nox2 complex. We have also demonstrated how Nox2 acts directly at the appressorium pore during its re-polarisation.

Roles of protective pigments in oxidative stress responses of the rock-inhabiting model fungus *Knufia petricola* A95. Nicole Knabe[1], Anna Gorbushina[1,2]. 1) BAM - Federal Institute for Materials Research & Testing, Department 4.1 (Biodeterioration and Reference Organisms) Berlin, Germany; 2) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy & Department of Earth Sciences, Berlin, Germany.

Rock-inhabiting microcolonial fungi (MCF) are able to colonise barren surfaces in almost every environment and are unequalled among eukaryotes in their ability to withstand extreme environmental conditions. Protective pigments, like melanin and carotenoids, have been proven to contribute to this unique robustness. An excellent model system to investigate the involvement of pigments in stress response and DNA repair is the MCF *Knufia petricola* strain A95. This non-pathogenic fungus possesses all characteristic features of MCF, including meristematic growth, melaninised cell-walls and extensive secondary metabolite production. This study is focusing on *K. petricola* responses to oxidative stress - one of the most significant environmental challenges encountered by MCF. The exact role of pigments and especially the interplay between carotenoids and melanin in the oxidative stress response is studied in wild type cells and constructed pigment knock-out mutants. Deletion of polyketide synthase in *K. petricola* (ΔPKS) leads to a complete loss of melanin, phenotypically revealing the carotenoids which are normally hidden beneath the melanised cell wall. In scytalone dehydratase mutants (ΔSDH) the melanin synthesis pathway is not disrupted completely and the colonies appear darker than ΔPKS. Additionally in ΔSDH phenotype precursors of DHN-melanin diffuse into the media causing the reddish-brown staining of the agar. In comparison to the wildtype strain, treatment of both melanin mutant strains with the oxidative agent H_2O_2 (up to 30 mM) shows no dose-dependent growth reduction. This indicates a relevant influence of the carotenoid pigments which has to be proven in further experiments. Comparative gene expression analyses concentrate on genes which are especially regulated under oxidative stress conditions and will help to elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

Identification and characterization of a NADPH oxidase target in *Fusarium graminearum*. Salima Chatur[1,2], Manisha Joshi[1], Chris Rampitsch[3], Li Wang[1], Gopal Subramaniam[1]. 1) AAFC 960 Carling Avenue Ottawa, ON Canada K1A 0C6; 2) Carleton University Department of Biology 1125 Colonel By Drive Ottawa, ON Canada K1S 5B6; 3) AAFC 101 Route 100 Morden, MB Canada R6M 1Y5.

F. graminearum is a fungal plant pathogen that causes Fusarium Head Blight (FHB) on important food and feed cereal crops including wheat, maize and barley. It is ranked as a major global plant pathogen causing significant yield reduction resulting in economic losses. Earlier study identified distinct roles for NADPH oxidase (NOX) genes in *F. graminearum.* NOX enzymes generate reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), which are important in signal transduction. The study indicated that while Δ*noxA* strain has reduced superoxide production and is unable to develop sexual fruiting bodies and ascospores, the Δ*noxA*/*B* double mutant has reduced pathogenicity on wheat. To elucidate the mechanism of NOX enzymes, an LC-MS approach was used to examine redox changes in the *ΔnoxA/B* proteome compared to wild-type *F. graminearum* strain. Samples were enriched for redox sensitive cysteine residue(s) and candidate proteins were identified via shotgun (gel free) linear ion trap mass spectrometery. Deletion analyses and overexpression of one of the candidate genes with modified cysteine residues confirmed that it is likely a genuine substrate of the NOX enzyme complex. Deletion of *Fg10089* as well as modification of this cysteine residue results in reduced virulence on wheat. However, no differences in the production of 15-A deoxynivalenol in culture is observed. The further characterization of this protein and its contribution to *F.graminearum* physiology and function will be examined.

The NADPH oxidase complex in *Botrytis cinerea* - New functions, members and the potential link to essential ER functions. Robert Marschall, Ulrike Siegmund, Paul Tudzynski. Institute of Plant Biology, University of Münster, Münster, Germany.

Reactive oxygen species (ROS) are produced in conserved cellular processes either as byproducts of the cellular respiration in mitochondria or as a support for defense mechanisms, signaling cascades or cell homeostasis. Their most common enzymatic producers are NADPH oxidases (Nox). In fungi several subunits of these complexes have been identified and were shown to be involved in sexual differentiation, pathogenicity and therewith in plant-fungi interactions.

Botrytis cinerea, also known as the gray mold fungus, is a necrotrophic plant pathogen with a broad host spectrum containing economically important crops like tomatoes and grapes. In this phytopathogenic fungus two NADPH oxidase isoforms (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified (Segmueller et al., 2008). *For B. cinerea* recently a new component was identified. The putative ER protein BcNoxD interacts directly with BcNoxA, and therewith presents for the first time a direct interaction between the catalytic subunits of the Nox-complex and other cellular components in fungi.

Deletion mutants of BcNoxA and BcNoxD display an identical phenotype regarding pathogenicity, sclerotia and conidia formation. Interestingly, both proteins seems to be not essential for the production of superoxide, since it was shown by the use of a new ROS detection agent, that the knock out has no effect on external ROS levels and superoxide production during the penetration of onion epidermal layers. Complementation studies revealed functions of the catalytic subunit BcNoxA inside the ER. Putative effects on the redox state can be displayed by the redox sensitive biosensor RoGFP2 as an efficient tool for the measurement of redox state changes (Heller et al., 2012).

Heller J, Meyer AJ, Tudzynski P (2012) Mol Plant Pathol. 13(8):935-47

Segmueller N. et al., (2008) Mol Plant Microbe Interact 21: 808-808-819.

Two new redox signaling proteins from *Podospora anserina*. P. Silar, H. Lalucque, F. Malagnac, P. Grognet, L. Chan Ho Tong. LIED, Univ Paris Diderot, Paris, France.

The genetic analysis of the cell degeneration Crippled Growth (CG) of *Podospora anserina* has been invaluable in the deciphering of the regulatory network controlling the entry into stationary phase, which include pigment, aerial hyphae and anastomosis formation, as well as proper set up of fruiting. Indeed, CG seems to stem from its misactivation in actively growing apices. Among the genes identified as crucial in the proper set up of CG and stationary phase are the MAP Kinase pathways PaMpk1 and PaMpk2, as well as the NADPH oxidase PaNox1. How the superoxide-generating PaNox1 is able to activate the MAP kinases is still not understood. Though a genetic screen we identified two genes that could encode proteins connecting these two components of the network. I will present what we know about these two genes and how they could act in the signaling pathway.

Saturday, March 21 2:00 PM–5:00 PM
Nautilus

Surface Growth in Filamentous Fungi and Yeast
Co-chairs: Peter Philippsen and Meritxell Riquelme

Function of the yeast exocyst complex in cell growth and division. Mary Munson[1], Margaret Heider[1], Mingyu Gu[4], Caroline Duffy[1], Zhanna Hakhverdyan[2], Michael Rout[2], Adam Frost[3,4]. 1) UMass Medical School, Worcester, MA; 2) The Rockefeller University, New York, NY; 3) University of California, San Francisco, CA; 4) University of Utah, Salt Lake City, UT.

Eukaryotic cells are crowded with membrane-bound vesicles that transport cargo between subcellular organelles, and to the plasma membrane for secretion. Mutations in proteins that regulate exocytosis result in accumulation of secretory vesicles that do not fuse, and defects in cell growth and division. Highly conserved machinery has evolved for spatial and temporal control of this multitude of membrane fusion events, but the molecular details of these processes are not well understood. SNARE proteins are core components of the membrane fusion machinery, and regulation of the SNAREs is crucial for the precise specificity and timing of fusion. Several protein families are critical for SNARE regulation, including large multisubunit tethering complexes, such as the exocyst complex at the plasma membrane. The exocyst is essential for cellular growth, secretion and endocytosis. It is a hetero-octameric protein complex localized to sites of secretion on the plasma membrane, where it is thought to function in quality control through specific tethering of secretory vesicles. Our earlier genetic, biochemical and phenotypic studies in *Saccharomyces cerevisiae* indicate functional cooperation between the exocyst complex, SNAREs, and the SNARE regulatory protein Sec1 to regulate the specificity and timing of SNARE complex assembly and membrane fusion. Further studies of the exocyst architecture and interactions with binding partners are facilitated by our recently developed purification strategy for obtaining native, intact yeast exocyst complexes. We are investigating its structure and stoichiometry using several approaches, including biochemical, genetic and proteolytic disruptions of the complex, mass spectrometry, chemical crosslinking, and binding studies with recombinant partners. By altering solution conditions or degrading specific subunits, we can purify novel exocyst subcomplexes that are likely to be important for assembly and function. Moreover, negative stain electron microscopy reveals our first view of the overall structure of the intact yeast exocyst complex.

Plasma membrane and cell wall expansion in *Neurospora crassa* hyphae. Meritxell Riquelme[1], Eddy Sánchez-León[1], Robert Roberson[2], Salomon Bartnicki-García[1], Gerhard Gierz[3]. 1) Department of Microbiology, CICESE, Ensenada, Baja California, 22860, Mexico; 2) School of Life Sciences, Arizona State University, Tempe, Arizona 85287, USA; 3) Department of Mathematics, University of California, Riverside, California 92521-0122, USA.

Fungal hyphae extend by tip growth, a mechanism that involves localized expansion of the plasma membrane and the cell wall. This process encompasses the polarized delivery of vesicles containing cell wall biosynthetic enzymes (CWBE) and/or precursors that will contribute to build new cell wall at the apex. In *Neurospora crassa*, before fusing with the plasma membrane, vesicles carrying CWBE concentrate at hyphal apices in a highly organized structure, the Spitzenkörper (Spk). We have found microvesicles (≈ 32 nm) containing chitin synthases (chitosomes) at the core of the Spk, whereas macrovesicles (≈ 83 nm) with β-1,3-glucan synthase activity occupy the outer layer of the Spk. Small Rab GTPases in their GTP-bound active state interact with the membrane of the vesicles and promote their association with tethering factors, before they fuse with a target membrane. In a mass spectrometry analysis of proteins that co-immunoprecipitated with CHS-1, CHS-4 and CHS-5, we identified YPT-1/Rab1, a Rab GTPase that regulates different secretory pathway steps in *Saccharomyces cerevisiae.* In *N. crassa* fluorescently tagged YPT-1 was found at early and late Golgi cisternae, and also at the microvesicular core of the Spk. In contrast SEC-4/Rab8 and YPT-31/Rab11 occupied the Spk macrovesicular peripheral layer, suggesting that the traffic and positioning of macrovesicles and microvesicles at the Spk is differentially regulated by distinct Rabs. FRAP analysis of the Rab GTPases and the CWBE at the Spk revealed low half-time recovery ($t_{1/2}$) values, indicating the existence of high rates of vesicles flowing into and out of the Spk, which maintains a steady state. The $t_{1/2}$ values found for Rabs (10-16 s) were lower than those for CWBE (21-32 s), suggesting a transient association of Rabs with the secretory vesicles. The data acquired experimentally is being used to test earlier predictions of vesicle flow rates needed to maintain the hyphal growth rates observed in *N. crassa*.

Maintenance of active directional growth by continual assembly and disassembly of polarity sites. Norio Takeshita. Dept. of Microbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany.

Polar cell extension depends on spatially defined insertion of new materials controlled by a group of cell-end marker proteins that are concentrated at the plasma membrane. In filamentous fungus *Aspergillus nidulans* , hyphal tips extend with speeds of 0.3-1.0 µm/min, requiring high frequency of exocytosis events (400-2000 vesicles/min). Here, the cell-end marker protein TeaR in *A. nidulans* is used to investigate the maintenance of polarized growth in the midst of rapid influx of vesicles by using a combination of fluorescence microscopy, a super-resolution microscopy technique (PALM) and computational modelling. We report that TeaR cluster is spatially dynamic and transiently stable. Accumulated TeaR triggers downstream processes of actin polymerization and active exocytosis, which results in localised cell extension and TeaR dispersion along the membrane. The reestablishment of polarity is driven by microtubules. These findings suggest a cyclic mechanism by which TeaR polarity is assembled and disassembled repeatedly to maintain polarity despite massive membrane flow.

Functional domains of the developmental regulator FlbB mediate the tip-to-nucleus communication in *Aspergillus nidulans* vegetative hyphae. Erika Herrero-García[2], Elixabet Perez de Naclares-Arregui[1], Marc S. Cortese[1], Ane Markina-Iñarrairaegui[1], Oier Etxebeste[1], Eduardo A. Espeso[2], Unai O. Ugalde[1]. 1) Biochemistry II Lab., Dept. of Applied Chemistry, The University of The Basque Country, 20018, San Sebastian, Gipuzkoa, Spain; 2) Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain.

Polar cells have developed multiple mechanisms to convey environmental signals from the polarity site to the nucleus and induce the appropriate cellular response. These mechanisms include transcription factors located at the polarity site, such as FlbB, which signals asexual development in vegetative hyphae of the filamentous fungus *Aspergillus nidulans*. FlbB is detected at the tip and apical (but not distal) nuclei, and understanding the relationship between these pools is crucial for the elucidation of the mechanisms that induce conidiation. Photo-convertible tagging with Dendra2 demonstrated a directionality of FlbB movement from the tip to nuclei, in a process that required an N-terminally located nuclear localization signal. Tip localization of a constitutively expressed GFP::FlbB chimera was abolished in the null mutant of its apical interactor FlbE, while the nuclear pool was increased. The aconidial phenotype of this strain demonstrated that tip processing of FlbB is a prerequisite for the induction of conidiation in nuclei. The bZIP domain of FlbB is essential and sufficient to enable the interaction with FlbE. However, the retention of FlbB at the tip also requires the C-terminal domain, since the substitution of the cysteine 382 by an alanine disrupted the apical localization. Overall, these findings demonstrate that fungal-specific adaptors and the establishment of a specific three dimensional conformation are key requirements for the apical localization of FlbB and demonstrate that nuclei are asymmetrically fed with a transcriptionally active pool originating at the tip.

Coordination between receptors, adaptors and the actin machinery at endocytic sites. Yidi Sun, David Drubin. University of California at Berkeley, Department of Molecular and Cell Biology.

Clathrin-mediated endocytosis can be divided into at least three steps of membrane remodeling: site initiation, vesicle budding and vesicle scission. Live cell imaging studies revealed that over 60 highly conserved proteins sequentially appear at endocytic sites in both budding yeast (*Saccharomyces cerevisiae*) and mammalian cells. Recent studies determined the precise localization of each endocytic protein along the invaginated endocytic membrane. Using this information, yeast endocytic proteins are classified into several modules: the early module, early coat module, coat module, WASP/MYOSIN module, actin module and amphiphysin module. The yeast endocyotic internalization process can be more simply divided into two stages, which are the early phase and the late phase. The proteins recruited during the early phase (including mostly early module and early coat module proteins) generally show longer and irregular life-times, while the proteins initiated at the late phase (including coat module, WASP/MYOSIN module, actin module and amphiphysin module proteins) show shorter and more regular life-times. The early phase proteins are believed to function in determining endocytic site location and in cargo recruitment. Many of the late phase proteins function in regulating the endocytic actin machinery, which is essential for endocytic vesicle formation in yeast and some types of mammalian cells. In budding yeast, Eps15-like proteins (Pan1 and End3) and a CIN85-like protein (Sla1) proteins arrive at the junction of the early and late phases, coincidently with arrival of the WASP (Las17) actin assembly regulator. Previous *in vitro* studies suggest that Pan1, End3 and Sla1 interact with each other and form a heterotrimeric complex. Pan1 and End3 are essential for cell growth and endocytic vesicle formation, respectively. However, the precise roles of these two proteins remained to be addressed. We used live cell imaging in parallel with genetics and biochemistry to investigate how Pan1, End3 and Sla1 are involved in endocytosis. Our results suggest a mechanism for how recruitment and activity of receptors (and cargo), adaptors and the actin machinery are coordinated at endocytic sites by Pan1, End3, and Sla1.

Polar growth and endocytosis in *Ashbya gossypii*. Doris Nordmann[1], Kumiko Masai[2], Peter Philippsen[2], Hans-Peter Schmitz[1]. 1) Department of Biology, University Osnabrück, Germany; 2) Biozentrum, University of Basel, Switzerland.

Polar surface expansion at the tip of hyphae is still poorly understood. Studies in several filamentous fungi have shown a spacial separation of exocytosis at the front of hyphal tips and endocytosis at the rim of hyphal tips. We and others hypothesized that polarity factors, exocyst components, and excess of membranes have to be internalized by endocytosis adjacent to the zone of extensive vesicle fusions in hyphal tips in order to maintain polar growth. We study endocytosis in *A. gossypii* which, based on its genome, is closely related to budding yeast but grows exclusively as multinucleated hyphae with up to 40 times faster surface growth rate compared to budding yeast. A comparative study of endocytic components in both organisms could therefore reveal essential hints to explain the differences in polar growth efficiency. In addition, it is very convenient that actin patches, which mark sites of endocytosis, can be stained in both organisms with Phalloidin, and that targeted gene manipulations work as efficiently in *A. gossypii* as in yeast. We could demonstrate that a streamlined endocytic process is essential for fast polar surface expansion in *A. gossypii*. Hyphal growth rates are decreased in 16 of 20 deletion mutants of endocytic genes. The other four deletions were lethal. The genes were selected from the 4 phases of endocytosis defined in yeast (Kaksonen and Drubin 2003, 2005). Null mutants of the yeast orthologs do not show the same pattern of reduced growth/lethality. In some of the slow growing *A. gossypii* mutants, the zone of endocytosis was no longer localized at the rim of hyphal tips. Next we investigated whether the faster surface growth potential of *A. gossypii* is reflected by faster endocytic events or by a higher density of endocytic events compared to yeast. Using Live-cell imaging Microscopy together with TIRF we monitored 9 fluorescently-labeled endocytic proteins in *A. gossypii* and found that some endocytic steps proceed up to 10 times faster as in yeast. Also, the number of endocytic events per square micrometer is significantly increased in *A. gossypii*. Finally, we tried to find an answer to the question why *A. gossypii* is lacking an ortholog of the important endocytic yeast gene SLA2.

CONCURRENT SESSION ABSTRACTS

Depletion of the mitotic kinase Cdc5p in *Candida albicans* results in the formation of elongated buds that switch to the hyphal fate over time in a Ume6p-dependent manner. Amandeep Glory[1], Chloë Triplet van Oostende[2], Anja Geitmann[2], Catherine Bachewich[1]. 1) Biology Department, Concordia University, Montreal, Quebec, Canada; 2) Institut de recherche en biologie végétale, Université de Montréal, Montreal QC, Canada.

The fungal pathogen *Candida albicans* differentiates between yeast, hyphae and pseudohyphae in order to enhance survival in the human host. Environmental cues induce hyphal development and expression of hyphal-specific genes. *C. albicans* can also form filaments in response to yeast cell cycle arrest, in the absence of environmental cues, but the nature of these cells and their mechanisms of formation are less clear. We previously demonstrated that depletion of the mitotic polo-like kinase Cdc5p resulted in the production of filaments under yeast growth conditions that were distinct from hyphae with respect to several criteria, yet maintained polarized growth and expressed hyphal-specific genes at later stages of development. In order to clarify the nature of these growth forms and their relationship to true hyphae, we conducted time course-based investigations of aspects of the polar growth machinery, which can help distinguish cell types. In Cdc5p-depleted cells, the Cdc42p GAP Rga2p became hyper-phosphorylated, as in true hyphae, but this was observed at only later stages of *CDC5* repression. Further, the hyphal-specific genes *HWP1*, *UME6* and *HGC1* were strongly induced at approximately the same time as Rga2p phosphorylation. The tips of some later-stage filaments also demonstrated the hyphal-specific Spitzenkörper-like localization of the myosin light chain Mlc1p. *HWP1* expression was dependent on Ume6p, and absence of Ume6p or Hgc1p influenced late-stage filament diameter and integrity. Finally, polarized growth and *UME6* expression in Cdc5p-depleted cells were independent of the transcription factor Hms1p. Thus, depleting Cdc5p may generate elongated yeast buds that switch to the hyphal fate over time through a mechanism that involves *UME6* induction, possibly in response to maintenance of polarized growth. The results expand on the multiple strategies with which *C. albicans* can modulate growth mode and expression of virulence determinants.

Assessing the role of exocytosis and endocytosis in fungal morphogenesis. Salomon Bartnicki-Garcia, Fernando Lara-Rojas, Rosa Mourino-Perez. Dept Microbiology, CICESE, Ensenada, Mexico.

The involvement of vesicles in the growth of fungal hyphae is well established but the complexity of the process leaves many unanswered questions. Whereas exocytosis is directly responsible for the growth of the cell wall and plasma membrane, the exact role of endocytosis has yet to be clearly defined. It is commonly assumed that exocytosis creates an excess of plasma membrane and thus the need for removal by endocytosis. This excess seems to be caused by the greater number of vesicles required for cell wall extension and extracellular enzyme secretion over the amount necessary for just plasma membrane extension. The highly localized processes of exocytosis (hyphal apex) and endocytosis (subapical collar) can be seen in living cells by following actin dynamics with Lifeact tagged hyphae. Estimates of membrane flow between exocytosis and endocytosis are difficult to calculate given the absence of reliable values for some critical parameters. Nevertheless, we have developed an interacting spreadsheet to examine the interplay of parameters for which actual data exist such as growth rate, cell shape and size, wall thickness and vesicle size. But in the absence of factual data for other critical factors such as amount of wall generated by each exocytic discharge, relative contribution of macro- vs. microvesicles, proportion of pre-formed cell wall vs. polymer synthesized in situ, and vesicle load destined for extracellular secretion vs wall formation, we have embodied them into a single factor: “vesicle packing efficiency”. Accordingly, using the best estimates for critical parameters, an excess of plasma membrane was always produced from exocytosis in a simulated hypha of *Neurospora crassa*. Experimental measurements of endocytosis were attempted by photobleaching the subapical endocytic collar of hyphae of *N. crassa* tagged with endocytic reporters fimbrin-GFP or coronin-GFP. The transient appearance of fluorescent patches, each indicative of an endocytic event, was monitored by confocal microscopy. Based on an estimated range of 50 - 80 nm for the diameter of endocytic vesicles, we recorded values indicating that 5 to 19% of the exocytosed membrane was endocytosed.

POSTER SESSION ABSTRACTS

Biochemistry and Metabolism

1. Biochemical characterization of a mitochondrial nicotinamide adenine dinucleotide carrier in *Aspergillus fumigatus*. L.L.L. Balico[1], F.G. Cardoso[2], S. Suzuki[2], E.S. Santos[1], S.A. Uyemura[1]. 1) FCFRP - USP, Ribeirão Preto, Brazil; 2) FMRP - USP, Ribeirão Preto, Brazil.

Aspergillus fumigatus is a saprophytic fungus and a major opportunistic pathogen in immunosuppressed patients. Our laboratory has previously shown that NAD^+ induces the formation of a membrane potential in *A. fumigatus* mitochondria, which could be dissipated by FCCP. This result suggested the presence of a NAD^+/NADH carrier in this fungus, as described in *S. cerevisiae*. Using bioinformatics tools, we were able to identify at the *Aspergillus* Genome Database, a sequence with 32% identity with *ndt1* gene from *S. cerevisiae*. In order to understand its function, that sequence from *A. fumigatus* was cloned and expressed in an eukaryotic model (*S. cerevisiae*). A cDNA fragment was amplified from the *ndt1* mRNA sequence and cloned in *pGEM®-T Easy* vector. For expression in *S. cerevisiae*, this sequence was subcloned in pYES2 vector and used to transform a *S. cerevisiae Δndt1Δndt2* strain. Recombinant protein expression was induced and after 16 hours it was detected by Western Blotting using a polyclonal anti-Ndt1 antibody. The growth curve in fermentable medium showed that the double mutant strain (control) grows in a slower rate compared to that strain expressing the recombinant protein. Mitochondria isolated from both strains were subjected to measurement of membrane potential and showed coupling between substrate oxidation and oxidative phosphorylation. Furthermore, NAD^+ induced a higher membrane potential in the strain expressing the recombinant protein than in control strain. Also, NAD^+ transport was evaluated by measurement of NADH fluorescence. Ndt1 expressing strain showed an increase in NADH fluorescence, indicating that NAD^+ was able to enter the mitochondrial matrix and reduced to NADH. Our results confirm the activity of Ndt1 as a mitochondrial nicotinamide adenine dinucleotide carrier.

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2. The proteinogenic and non proteinogenic function of histidine in *Aspergillus fumigatus*. Anna-Maria Dietl[1], Nicola Beckmann[1], Ulrike Binder[2], Jorge Amich[3], Sixto Leal[4], Eric Pearlman[4], Hubertus Haas[1]. 1) Division of Molecular Biology, Medical University Innsbruck, Tyrol, Austria; 2) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Tyrol, Austria; 3) Medical University Würzburg, Germany; 4) Case Western Reserve University, Ohio, United States of America.

Aspergillus fumigatus is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from non-specific symptoms of infection, poor diagnostics and comparatively few options for treatment. Novel antifungal therapy approaches target fungal-specific pathways that are essential for virulence. One potential example is the biosynthesis of the essential amino acid histidine. Bacteria, plants und fungi produce histidine via a highly conserved pathway, in *A. fumigatus* encoded by eight genes. In contrast, animals and humans do not synthesize histidine and satisfy their demand via nutritional uptake. Here we demonstrate that lack of histidine biosynthesis due to genetic abrogation of the gene encoding imidazoleglycerol-phosphate dehydratase (HisB) causes histidine auxotrophy and virulence attenuation in four virulence models: *Galleria mellonella*, murine pulmonary aspergillosis, murine systemic infection and murine keratitis. In agreement with the *in vivo* importance of histidine biosynthesis, the HisB inhibitor 3-amino-1,2,4-triazol (3-AT) reduced the virulence of the *A. fumigatus* wildtype in *Galleria mellonella*. In line with a crucial role of histidine in cellular handling of metals due to its chelator activity, HisB-deficiency decreased the resistance of *A. fumigatus* to a variety of metals including iron, zinc, nickel, cobalt, copper and manganese. Taken together, this study reveals (i) limited histidine availability in different *A. fumigatus* host niches and (ii) the histidine biosynthetic pathway as target for novel antifungal therapy approaches.

3. Fumigatin-oxide production by *Aspergillus fumigatus* is regulated by iron availability and temperature involving the transcription factors HapX and SrbA. Beatrix E. Lechner[1], Ernst R. Werner[2], Markus A. Keller[2], Kirstin Scherlach[3], Falk Hillmann[4], Hubertus Haas[1]. 1) Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria; 2) Division of Biological Chemistry, Innsbruck Medical University, Innsbruck, Austria; 3) Department of Biomolecular Chemistry, HKI, Jena, Germany; 4) Department of Molecular and Applied Microbiology, HKI, Jena, Germany.

Iron is an essential metal for the metabolism of virtually all species. For the opportunistic fungal pathogen *Aspergillus fumigatus*, adaptation to iron starvation has been shown to be an essential virulence determinant. Here we found that in *A. fumigatus* liquid cultures, iron starvation induces the secretion of a yellow pigment optimally at 20-25°C, but not at 37°C, within 48-72 h of growth. In contrast, starvation for nitrogen, carbon, phosphate or other metals such as copper or zinc did not trigger production of this extracellular pigment. Deficiency in HapX or SrbA, the master regulators for adaptation to iron starvation and secondary metabolism, respectively, impaired biosynthesis of the pigment. In contrast to *A. fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus* or *Penicillium chrysogenum* were not found to synthesize this pigment. The pigment was purified by solid phase extraction and reversed-phase HPLC separations. High-resolution mass spectrometry revealed a molecular mass of 183.0297 corresponding to the chemical formula of $C_8H_8O_5$. 1H-NMR together with the photosensitivity and the pH dependence of UV absorption spectra identified the compound as fumigatin-oxide. The aromatic, maroon-colored metabolite fumigatin, showing antibiotic activity against bacteria and toxicity against animals as well as anti-inflammatory activity, was first isolated from *A. fumigatus* culture media in 1938. Subsequently, fumigatin and its derivatives were detected by mass spectrometry in various *A. fumigatus* isolates but the biosynthetic pathway for fumigatin biosynthesis remains to be elucidated. This study represents the first characterization of the regulation of fumigatin production and emphasizes the impact of iron availability on fungal secondary metabolism.

4. The MFS transporter gene in GT biosynthetic cluster is necessary for GT secretion and protection. Elizabeth Smith, Stephen Hammel, Sean Doyle, Grainne O' Keeffe, Gary Jones. Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland.

Gliotoxin is a non ribosomally synthesized metabolite, secreted *by Aspergillus fumigatus.* It is an epipolythiodioxopiperazine (ETP) which is characterised by an intramolecular disulphide bridge. The *gli* gene cluster is comprised of thirteen genes which are involved in aspects of gliotoxin biosynthesis and self-protection. The cluster includes *gliA* (CADRE locus identifier: AFUA_ 6G09710), a gene which is predicted to encode a transmembrane gliotoxin efflux pump, which is a member of the Major Facilitator Superfamily (MFS). *A. fumigatus* also secretes an inactive bis-S-methylated form of gliotoxin (BmGT). The role of *gliA* in the biosynthesis and secretion of gliotoxin by *A. fumigatus* is unknown, however a previous study in which the *sirA* gene, an ortholog of *gliA*, from *Leptosphaeria maculans* was deleted, resulted in increased sensitivity to both gliotoxin and sirodesmin, and also an increase the secretion of sirodesmin from *L. maculans*. Deletion of *gliA* was undertaken in *A. fumigatus* ATCC26933, previously shown to produce gliotoxin at high levels, using a split marker strategy and acquisition of pyrithiamine resistance. Both Southern and qRT-PCR analysis confirmed deletion of *gliA* and absence of *gliA* expression in *A. fumigatus* $\Delta gliA^{26933}$, respectively. Deletion of *gliA* completely abolished gliotoxin secretion, as determined by both RP-HPLC and LC-MS analysis, compared to that from *A. fumigatus* ATCC26933. Interestingly, secretion of the gliotoxin derivative, BmGT was not inhibited, indeed, there was a significant increase in the levels of BmGT secreted by *A. fumigatus* $\Delta gliA^{26933}$ compared to wild type between 48-96 h growth ($p < 0.001$). Exposure of *A. fumigatus* $\Delta gliA^{26933}$ to exogenous gliotoxin revealed a significantly ($p < 0.001$) sensitive phenotype, compared to wild type. These results strongly suggest a role for *gliA* in the secretion of endogenously produced gliotoxin, but not bis-methyl gliotoxin, and that *gliA* functionality is necessary to protect against exogenous gliotoxin.

5. Commonalities and partial redundancy of two LaeA- and BrlA-regulated conidial polyketide metabolites in *A. fumigatus*. Kurt Throckmorton[1], Fang Yun Lim[2], Dimitrios Kontoyiannis[3], Weifa Zheng[4], Nancy Keller[2]. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) MD Anderson Cancer Center, University of Texas, Houston, TX; 4) Key Laboratory for Biotechnology, Jiangsu Normal University, Xuzhou, China.

The opportunistic human pathogen *Aspergillus fumigatus* produces many secondary metabolites, some of which are immunomodulatory or toxic and are thought to contribute to its virulence. Two such secondary metabolites, trypacidin and endocrocin, are both produced by non-reducing polyketide synthases, and are localized to the conidium of *A. fumigatus*, requiring LaeA and BrlA for their synthesis. Furthermore, they are predicted to share an early precursor, atrochrysone carboxylic acid, and are both thermally regulated, being produced at lower levels at higher temperatures. In an isolate of *A. fumigatus* which does not produce trypacidin, CEA10, deletion of the endocrocin polyketide synthase results in loss of endocrocin. However, in an isolate which produces trypacidin, AF293, the same deletion does not affect production of endocrocin. Genetic dissection of the biosynthetic pathways of these metabolites suggests that endocrocin is produced by both of these physically discrete clusters. This redundancy is of uncertain adaptive advantage, but is the first example of its kind.

6. Multiple phosphatases regulate carbon source dependent germination and primary metabolism in *Aspergillus nidulans*. Leandro Assis[1], Laure Ries[1], Marcela Savoldi[1], Taisa Dinamarco[1], Neil Brown[1], Gustavo Goldman[1,2]. 1) Molecular Biology, FCFRP - USP, Ribeirão Preto, São Paulo, Brazil; 2) Lab. Nacional de Tecnologia do Bioetanol, CTBE-CNPEM, Campinas, São Paulo, Brazil.

The morphological and biochemical transition from dormant conidia into active, growing, filamentous hyphae requires the coordination of numerous biosynthetic, developmental and metabolic processes. The present study demonstrated the diversity of roles performed by seven phosphatases in *Aspergillus nidulans*. Seven NPP (non-essential protein phosphatases) null mutants were shown to be unable to grow directly on glucose, xylose, glycerol, ethanol and acetate as sole carbon sources, but were able to grow directly on hydrolyzed casein (CA) or tributyrin and media containing both CA and glucose. This suggested that the seven NPP mutants possessed defects in the sensing and/or metabolism of carbon sources which entered primary carbon metabolism, but were able to grow on amino acids and lipids, which entered primary carbon metabolism as TCA cycle intermediates. The impact of seven NPP in the glucose-dependent breaking of conidial dormancy and produce isotropic growth in glucose containing media was assessed. One mutant was unable to induce swelling suggesting a major defect in the detection of glucose, while three mutants also demonstrated a significant reduction in conidial swelling. Another three mutants showed a significant increase in conidial swelling (>75%) and were therefore unlikely to possess major defects in glucose detection. Mapping the block in glucose metabolism reveals TCA cycle defects. The NPP mutants were able to utilize amino acids which acted as precursors for pyruvate, succinyl-CoA, fumarate and oxalacetate, but were unable to utilize amino acid precursors for acetyl-CoA or α-ketoglutarate. This suggested that the block in glucose metabolism was in the oxidative decarboxylation of α-ketoglutarate to succinyl-CoA. These novel insights into the fundamental roles of numerous phosphatases in germination and carbon sensing have provided new avenues of research into the identification of inhibitors of fungal germination, with implications for the food, feed and pharmaceutical industries.

7. Lactose transport in *Aspergillus nidulans*: Identification and expression of a second permease gene. Erzsébet Fekete, Anita Orosz, László Kulcsár, Michel Flipphi, Levente Karaffa. Biochemical Engineering, University of Debrecen, Debrecen, Hungary.

Lactose (milk sugar) is the main carbohydrate in whey, an abundant high-energy dairy residue. Yet, for most micro-organisms that can convert it (into glucose and galactose), it is a gratuitous carbon source that is slowly assimilated. To optimalise fermentation processes that use whey residue, and to further its use in second-generation biofuel generation and its removal from contaminated soil and water (bioremediation), we study lactose catabolism in the industrial cell factory *Penicillium chrysogenum* and the genetic model *Aspergillus nidulans*, a soil-borne saprophyte. Recently, we characterised the lactose permease LacpA, responsible for a considerable part of the prevalent uptake in *A. nidulans*, and showed that uptake rather than hydrolysis is the limiting step in its lactose catabolism (1). We now have identified a second physiologically relevant lactose transporter, LacpB. Glycerol-grown mycelia from mutants deleted for both *lacpA* and *lacpB* only appear to take up minute amounts of lactose. Moreover, mycelia of the double deletant strains appear unable to produce

new biomass from lactose. Although transcription of both *lacp* genes was strongly induced by lactose in pregrown wild-type mycelia, their inducer profiles differ markedly. *lacpB* responded also strongly to *beta*-linked glucopyranose dimers, cellobiose and sophorose, while these inducers of the cellulolytic system did not provoke any *lacpA* response. Nevertheless, *lacpA*/*B* double mutants grew like wild type on cellobiose which suggests that, in contrast to the situation with lactose, efficient cellobiose uptake in *A. nidulans* is mediated by third systems that make *lacpB* functionally redundant. We shall also report on currently ongoing studies with single *lacpB* deletion mutants and on the interplay of the inductory circuits that allow uptake and assimilation of lactose.

(1) Fekete *et al*., Fungal Genet Biol 49: 215-225.

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8. *Aspergillus nidulans* as cell factory for production of mycophenolic acid. Zofia D. Jarczynska[1], Jakob B. Nielsen[1], Freja Aasted[1], Dorte M. K. Holm[2], Kiran R. Patil[3], Kristian F. Nielsen[1], Uffe H. Mortensen[1]. 1) Systems Biology, DTU, Kgs. Lyngby, Denmark; 2) Novozymes A/S, Denmark; 3) European Molecular Biology Laboratory, Heidelberg, Germany.

Filamentous fungi are well-known producers of a wide range of valuable secondary metabolites (SMs), which can be advantageously exploited e.g. in pharmaceutical industry. One of the most prominent examples is mycophenolic acid (MPA), an immunosuppressant molecule that inhibits inosine-5'-monophosphate dehydrogenase (IMPDH). IMPDH catalyzes the rate limiting step in the guanine nucleotide synthesis in B- and T-lymphocytes. Recent studies have successfully identified the gene cluster, coding for the MPA synthesis, in *Penicillium brevicompactum*. Moreover, it has been demonstrated that two first steps in MPA production are catalysed, respectively, by polyketide synthase (PKS), MpaC, producing 5-methylorsellinic acid (5-MOA), and MpaDE, which strikingly is a natural fusion enzyme catalysing the production of 5,7-dihydroxy-4-methylphtalide (DHMP). Additionally, *mpaF* has been characterized as IMPDH-encoding gene which confers the resistance to MPA. In order to characterize the remaining part of the MPA biosynthetic pathway, we have heterologously expressed the *mpa* cluster genes in a stepwise manner in *Aspergillus nidulans*. We have demonstrated that MpaA possesses prenyl transferase activity and catalyzes the conversion from DHMP to 6-farnesyl-5,7-dihydroxy-4-methylphtalide (FDHMP). To our surprise, this strain was also able to produce demethyl-MPA, which is the next intermediate in MPA biosynthesis. Interestingly, we have found two homologs of *mpaH* in *A. nidulans*, which is hypothesized to encode the conversion of FDHMP to demethyl-MPA, and we speculate that one or both of these genes deliver hydrolase activity similar to the one encoded by MpaH. Lastly, we have confirmed that MpaH and MpaG catalyze the last two enzymatic steps in the biosynthesis of MPA, resulting in the production of demethyl-MPA and MPA, respectively. In conclusion, we have successfully characterized the full biosynthetic pathway of the top-selling drug, MPA. Moreover, we have demonstrated that *A. nidulans* is a suitable cell factory for heterologous production of MPA.

9. Promiscuity runs in the family - Analysis of nidulanins. Jakob B. Nielsen, Andreas Klitgaard, Maria L. Nielsen, Thomas O. Larsen, Mikael R. Andersen, Kristian F. Nielsen, Uffe H. Mortensen. DTU Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

Non-ribosomal peptides (NRPs) constitute a considerable group of secondary metabolites. These products are synthesized by large modular enzymes, the non-ribosomal peptide synthetases (NRPS). The selection of the amino acids incorporated in the NRP is decided by the adenylation domain(s) in the NRPS. The genome of *Aspergillus nidulans* encodes 14 putative NRPSs, and nearly as many pseudo NRPSs, NRPS-like enzymes, where some transfer a single amino acid to another moiety. Through gene expression analysis and deletions, we recently linked production of the prenylated cyclo-tetrapeptide nidulanin A, Phe-Kyn-Val-Val, to the activity of the NRPS AN1242 and the prenyl transferase AN11080. Through feeding experiments by stable isotope labeled amino acids (SILAAs), we observed that this particular NRPS is highly promiscuous. At least the two first adenylation domains allow incorporation of more than one type of amino acid to yield at least 8 other tetrapeptides including fungisporin, not previously described from *A. nidulans*. Strikingly, analysis of synteny for the locus of AN1242 in many of the sequenced *Aspergillus* and *Penicillium* species revealed extensive homology, among others *pes1* from *Aspergillus fumigatus* and *hcpA* of *Penicillium chrysogenum*. This was further verified by UHPLC-qTOFMS that detected fungisporins in all of 20 *Penicillium* species as well as 15 *Aspergillus* species analyzed. This cocktail of tetrapeptides synthetized from one NRPS and the presence of the gene cluster in so many species is intriguing and points to a central biological function of nidulanins.

10. A biocombinatorial engineering approach for production of novel synthetic natural products. Maria Lund Nielsen, Jakob B. Nielsen, Uffe H. Mortensen, Mikael R. Andersen, Thomas O. Larsen. Systems Biology, DTU, Kgs. Lyngby, Denmark.

Fungal secondary metabolism is the source of a large number of structurally diverse natural products that hold a wide variety of biological activities. Many of these compounds are known to possess activities of medical relevance, such as anti-bacterial-, anti-cancer-, cholesterol-lowering- and immunosuppressive. The need for new bioactive compounds calls for an approach to further expand the diversity of fungal secondary metabolites. One such approach is the biocombinatorial synthesis of novel natural products through the engineering of proteins involved in secondary metabolism. In particular, engineering of polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs) and PKS-NRPS hybrid proteins has been attempted; however, so far this approach has obtained only limited success. In order to fully exploit the possibilities of combinatorial biosynthesis we first need to understand the rules governing product formation. Such understanding would enable the rational design of functional chimeric enzymes and permit the synthesis of derivatives of known natural products. The PKS-NRPS hybrids have received particular interest as targets for combinatorial biosynthesis due to the potential of creating novel compounds of mixed polyketide-non-ribosomal peptide origin. One critical step in designing functional PKS-NRPS fusions is undoubtedly the determination of the optimal site for linking the two parts of the chimera.

In this study we focused on the PKS-NRPS from *Aspergillus clavatus* involved in the synthesis of the potential anti-cancer compound cytochalasin E. This enzyme appears to contain an intermodular linker of approximately 150 amino acids with very low sequence homology to other known PKS-NRPS hybrids. We investigated the role of this linker by constructing a number of linker-modified variants of the *A. clavatus* PKS-NRPS hybrid, including linker swaps, linker truncations and synthetic linkers. These experiments will help to unveil the importance of the linker, and facilitate determination of the optimal length of the intermodular linker, along with a suitable site for linking the two modules.

11. G protein-coupled receptor mediates nutrient sensing and developmental control in *Aspergillus nidulans*. Thaila Reis[1], Neil Andrew Brown[1], Enyara Rezende Morais[1], Lizziane K Winkelströter[1], Camila Caldana[2], Jae-Hyung Mah[3], Jae-Hyuk Yu[4], Jeffrey M Macdonald[5], Gustavo Henrique Goldman[1,2]. 1) University of Sao Paulo, Ribeirao Preto, Brazil; 2) Laboratório Nacional de Ciência e Tecnologia do Bioetanol - CTBE, Campinas, Brazil; 3) Department of Food and Biotechnology, Korea University, Sejong, South Korea; 4) Department of Bacteriology, University of Wisconsin, Madison, Wisconsin, USA;; 5) UNC Metabolomic Facility, University of North Carolina,Chapel Hill, USA.

Nutrient sensing and utilization is fundamental for all life forms. As heterotrophs, fungi have evolved with a diverse range of mechanisms for sensing and taking up various nutrients. Despite its importance, only a limited number of nutrient receptors and their corresponding ligands have been identified in fungi. G protein-coupled receptors (GPCRs) are the largest family of transmembrane receptors, which detect predominately unknown extracellular signals and initiate intracellular signalling cascades. In fungi, GPCR regulated signalling pathways include the cAMP-dependent protein kinase (PKA) and the mitogen-activated protein kinase (MAPK) cascades, which regulate metabolism, growth, morphogenesis, mating, stress responses and virulence. The *A. nidulans* genome encodes 16 putative GPCRs, but only a few have been functionally characterized. Our previous study showed the increased expression of an uncharacterised putative GPCR, *gprH*, during carbon starvation. Here, we reveal that GprH is a putative receptor for glucose and absence of GprH results in a reduction in cAMP levels and PKA activity upon adding glucose to starved cells. GprH is pre-formed in conidia and is increasingly active during carbon starvation, where it plays a role in glucose uptake and the recovery of hyphal growth. GprH also represses sexual development under conditions favouring sexual fruiting and during carbon starvation in submerged cultures. In summary, the GprH sensing system for glucose acts upstream of the cAMP-PKA pathway, influences primary metabolism and hyphal growth, while repressing sexual development in *A. nidulans*.

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12. Influence of microgravity on the production of *Aspergillus nidulans* secondary metabolites onboard the International Space Station. Clay Wang[1], Kasthuri Venkateswaran[2], Junko Yaegashi[1], Jillian Romsdahl[1]. 1) Pharma Sci & Chemistry, Univ Southern California, Los Angeles, CA; 2) Jet Propulsion Laboratory, Pasadena, CA.

In this poster I will present our project recently funded by the NASA Space Biology program to study the production of secondary metabolites by *Aspergillus nidulans* onboard the International Space Station. Research from the Wang lab and many others in the field have shown that in filamentous fungi secondary metabolite production is highly sensitive to growth conditions. In addition it has been shown that many secondary metabolism pathways are triggered specifically in harsh or stressful conditions. Therefore we are interested in understanding how filamentous fungi respond to microgravity conditions. There are two long term goals for this project. One is to discover novel secondary metabolites in microgravity conditions. Second is to develop filamentous fungi as a synthetic biology platform for producing pharmaceutical compounds for future manned space mission. In addition I will present data on our metabolite analysis of fungi recovered from the International Space Station Filter Debri as part of the NASA funded ISS microbial observatory project.

13. Exploring the potential of *Aspergillus niger* as secondary metabolite producer. Simon Boecker[1], Sophia Zobel[2], David Schirmer[2], Roderich D Suessmuth[2], Vera Meyer[1]. 1) Dept. of Applied and Molecular Microbiology, Berlin Institute of Technology, Berlin, Germany; 2) Dept. of Biological Chemistry, Berlin Institute of Technology, Berlin, Germany.

Recently, we could show that *Aspergillus niger* is an excellent expression host for the production of fungal secondary metabolites (SMs).[1] For the proof-of-concept study, we heterologously expressed the 350 kDa non-ribosomal enniatin synthetase (ESYN) from *Fusarium oxysporum* in *A. niger*. ESYN catalyzes the formation of cyclic depsipeptides of the enniatin family, which exhibit antimicrobial, antiviral and anticancer activities. The encoding gene *esyn1* was put under control of the tunable Tet-On expression system. By using optimum cultivation and feeding conditions, yields up to 4.5 g L^{-1} were achieved in *A. niger* fed batch bioreactor cultivation. This titer by far outpaces yields obtained in other microbial expression hosts, such as *B. subtilis*, which produces around 1 mg L^{-1} of enniatin.[2]

In addition, another cyclohexadepsipeptide (beauvericin) with anticancer and insecticidal properties can be produced with comparable high titers in *A. niger*. Furthermore, *A. niger* is a suitable expression host for new-to-nature artificial chimeric peptide synthetases. Corresponding data will be shown.

[1] **Richter L, Wanka F, Boecker S, Storm D, Kurt T, Vural Ö, Süßmuth R, Meyer V**. 2014. Engineering of *Aspergillus niger* for the production of secondary metabolites. Fungal Biol. Biotechnol. **1**:4.

[2] **Zobel S, Kumpfmüller J, Süssmuth RD, Schweder T**. 2014. *Bacillus subtilis* as heterologous host for the secretory production of the non-ribosomal cyclodepsipeptide enniatin. Appl. Microbiol. Biotechnol. (Epub ahead of print).

14. Development of a community consensus model for *Aspergillus niger*. Julian Brandl[1], Mhairi Workman[1], Mikko Arvas[2], Vera Meyer[3], Mikael R. Andersen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) VTT Technical Research Centre of Finland, Finland; 3) Department of Applied and Molecular Microbiology, Institute of Biotechnology, Technical University of Berlin, Germany.

Fungal primary metabolism is an essential part of fungal physiology and affects all phenotypic traits of the organism as well as carrying the biotechnological potential for the fungal host. While the study of individual pathways have gained essential knowledge and important scientific breakthroughs, a genome-scale view of metabolism is required to gain a holistic understanding of the cell. Mathematical models based on the stoichiometry of known enzymatic reactions have been developed in order to facilitate this approach and proven useful for guiding metabolic engineering in well characterized model organisms like *S. cerevisiae* and *E. coli.* With the sustained interest in *Aspergillus niger* as a potent host organism for citric acid and enzyme production, it is timely to improve on previous genome-scale modeling efforts. Here we aim at updating the genome-scale model by a combination of experimental work and integration of published information. This joint effort of international collaborators and our group will yield a community-consensus model of the *Aspergillus niger* metabolism. In order to improve the gene assignments contained in the current version of the model, we will use comparative genomics to identify shared isoenzymes and gene groups between closely related species in the section *Nigri.* To accurately examine and model the catabolic potential of the fungus, we will apply Biolog plates for the screening of more than 270 carbon and nitrogen sources. This knowledge will aid to identify missing pathways in the model and validate the presence of many pathways already included. Additionally the biosynthesis of 2-300 secreted enzymes will be included in the new version of the model. In conclusion this project aims at generating an experimentally validated community-consensus model of the *A. niger* metabolism being able to describe and predict beneficial modifications to the metabolic network in order to improve protein production on a variety of different substrates.

15. Heterologous Expression of Cellulases in *Aspergillus niger*. S.A. Campen[1,2], S.J. Sibert[2,3,5], S. Srikrishnan[1,2], J. Lynn[1,2], P. Phatale[1,2], J. Zhang[2,4], J.M. Guenther[2,4], T. Feldman[2,4], J. Hiras[2,5], S. Singer[2,5], K.L. Sale[2,4], B.A. Simmons[2,4], S.E. Baker[1,2], J.M. Gladden[2,4], J.K. Magnuson[1,2]. 1) Pacific Northwest National Laboratory, Richland, WA; 2) Joint BioEnergy Institute, Emeryville, CA; 3) University of California-Berkeley, Berkeley, CA; 4) Sandia National Laboratory, Livermore, CA; 5) Lawrence Berkeley National Laboratory, Berkeley, CA.

Deconstruction of lignocellulosic biomass is one of the challenges to development of advanced biofuel production. Pretreating the biomass with ionic liquids helps subsequent enzymatic hydrolysis of cellulose to sugar. Thermophilic bacterial cellulose-degrading enzymes have been previously identified from a switchgrass adopted microbial community and characterized within the Deconstruction Division of JBEI. Filamentous fungi, for example,*Aspergillus niger*, have been utilized for commercial cellulase production in industry. *Aspergillus niger* is an ascomycete filamentous fungus with a sequenced genome and is known to secrete various enzymes. Our objectives are to understand the production of heterologous enzymes in *A. niger* and further its development as a heterologous expression host for high titer cellulase cocktails. Thirty-two bacterial and fungal genes, encoding beta-glucosidases, cellobiohydrolases and endoglucanases with high temperature optima were expressed in *A. niger* and their enzyme activities were assayed. Using Jsalsa (the JBEI suite for automated lignocellulosic saccharification), the activity profiles with respect to temperature, pH and ionic liquid concentration were analyzed and compared for the enzymes expressed in *A. niger* and *E.coli*. These enzymes will be used to investigate improvements to JBEI's JTherm cellulase cocktail.

16. Biological role and characterization of aegerolysins and proteins with MACPF domain in filamentous fungus *Aspergillus niger*. Marusa Novak[1], Urska Cepin[2,3], Nada Krasevec[4], Peter Macek[1], Gregor Anderluh[4], Kristina Sepcic[1]. 1) University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia; 2) National Institute of Biology, Ljubljana, Slovenia; 3) BioSistemika Ltd, Ljubljana, Slovenia; 4) L11, National Institute of Chemistry, Ljubljana, Slovenia.

Aegerolysins and MACPF domain-containing proteins (Pfam06355 and 01823 protein families, respectively) are found in various kingdoms of life including fungi. In Basidiomycota, proteins of these two families seem to be involved in development of primordia and fruiting bodies, while in filamentous fungi they can also act as virulence factors. Various fungal members of both protein families have been shown to form pores in biological and artificial lipid membranes, either sole or in combination with one another. It appears that the roles of these proteins are pleiotropic and adapted to the fungal life-style.

Aspergillus niger is a saprophytic, filamentous fungus found throughout the world. In its genome, we identified two nucleotide sequences encoding aegerolysins and two nucleotide sequences encoding proteins with MACPF domain. Our aim is to determine the biological role(s) and some characteristics of these proteins in *A. niger* using systematic gene expression studies, gene deletions and protein labeling. So far, we showed that the increase of the expression of all four target genes coincides with the beginning of conidiation in *A. niger*, and that the prevention of conidiation (either physical or genetic) alters the expression profiles and negatively affects their expression. Deletion of either of the aegerolysin genes did not affect the rate of conidiation or growth on different media. Moreover, the localization studies using fluorescently labeled proteins showed proteins to be localized in hyphae, conidiophores heads and spores. Our results suggest that aegerolysins and MACPF domain-containing proteins are produced during conidiation of *A. niger*, but are not actively involved in this process, indicating that their role(s) might be related to some other physiological processes in the fungus, e.g. defense mechanisms, rather than development.

17. Reconstruction of the biosynthetic pathway for the terpene antibiotic pleuromutilin in the secondary host *Aspergillus oryzae*. Fabrizio Alberti[1], Colin M. Lazarus[1], Chris L. Willis[2], Andy M. Bailey[1], Gary D. Foster[1]. 1) School of Biological Sciences, University of Bristol, Bristol, United Kingdom; 2) School of Chemistry, University of Bristol, Bristol, United Kingdom.

Pleuromutilin is an antibiotic that is produced as a secondary metabolite by the basidiomycete fungus *Clitopilus passeckerianus*. Pleuromutilin is a tricyclic diterpene and has been exploited as a precursor for many semi-synthetic antibiotics, one of which –

Retapamulin – is currently used for the treatment of Impetigo and other serious skin infections. However *C. passeckerianus* produces pleuromutilin in low amounts, strain improvement and manipulation of the fungus are made problematic by its dikaryotic nature, and total synthesis of the antibiotic has only been achieved with low yields.

In order to increase the production titre of pleuromutilin and to fully exploit this new growing class of natural product antibiotics, the biosynthetic pathway for pleuromutilin was reconstructed in the heterologous host *A. oryzae*, which has a GRAS status and is amenable to growth in industrial fermenters. Multigene expression vectors were used to obtain transformation of *A. oryzae* with the genes of the pleuromutilin cluster. Identification of the metabolites produced was achieved through analytical chemistry techniques, such as HPLC, NMR Spectroscopy and High Resolution Mass Spectrometry.

A synthetic biology approach was used to achieve heterologous biosynthesis of pleuromutilin in *A. oryzae*. An enhanced titre of the antibiotic was ultimately established in the heterologous host, tenfold over the natural host producer *C. passeckerianus*. The function of each enzyme in the pathway is being uncovered through expression of different combinations of genes in *A. oryzae* and consequent isolation of metabolites. This strategy also allowed to isolate a previously undescribed intermediate involved in biosynthesis of the antibiotic pleuromutilin.

18. Investigating torrubiellone A gene cluster from *Torrubiella sp. BCC2165* in *A. oryzae* as heterologous host. G. Fernandez Bunster, C.M. Lazarus. Life Sciences Building, School of Biological Sciences, University of Bristol, Bristol, United Kingdom.

Torrubiellones A-D, extracted from *Torrubiella* sp. BCC2165 (Isaka *et al*, 2010), are structurally similar to 2-pyridone compounds produced in related arthropod-pathogenic fungi, such as tenellin and desmethylbassianin in *Beauveria* spp. and militarinone in *Cordyceps militaris*. Torrubiellone A is particularly interesting because it has antimalarial activity (IC50 value of 8.1 µM), with very weak accompanying cytotoxicity. Having studied the gene clusters responsible for the biosynthesis of the related compounds we predicted that sequencing the *Torrubiella* genome and *in-silico* analysis should lead to the identification of the torrubiellone A biosynthetic gene cluster.

Torrubiella BCC2165 DNA was extracted, sequenced and analysed using antiSMASH software, to reveal a putative torrubiellone A gene cluster, i.e. one encoding a hybrid PKS-NRPS (named *torS*), two P450 cytochromes (*torA* and *torB*) and an enoyl reductase (*torC*). By comparison to the tenellin and desmethylbassianin gene clusters, two additional genes (named *torD* and *torE)*, were also identified within the cluster; these genes may be responsible for structural differences between torrubiellone and desmethylbassianin. The PKS-NRPS gene (*torS* fused to *eGFP*) was assembled without introns by yeast recombination and put on a multigene expression vector with other biosynthetic genes either from the putative torrubiellone cluster (*torA, torB* and *torC*), or from the desmethylbassianin gene cluster (*dmbA, dmbB* and *dmbC*). The assembled plasmids were transferred into the filamentous fungus *Aspergillus oryzae* NSAR1, and both the *torSABC* and *torSdmbABC* plasmids yielded strongly yellow-pigmented transformants; organic extracts were analysed by liquid chromatography-mass spectroscopy (LC-MS). At the same time, *torD* and *torE* gene functions were investigated by coexpressing these genes in a tenellin-producing *A. oryzae* transformant; a product with a mass of 384, 15 more than the tenellin mass of 369, was detected.

19. Phenotype analysis of *Rice koji* protein genes disruptants in *Aspergillus oryzae*. Kazuhiro Iwashita[1,2], Teruaki Hanada[1,2], Shinichiro Hukuhara[1,2], Shingo Kakiuchi[1,2], Hiroaki Kitamura[1,2], Ken Oda[1], Minori Kouno[1]. 1) National Research Institute of Brewing, Higashihiroshima, Hiroshima, Japan; 2) Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashihiroshima, Hiroshima, Japan.

Aspergillus oryzae are used for Japanese traditional fermentation industry, such as Sake, Miso-paste, and Rice vinegar. In sake industry, *A. oryzae* cultivate on rice to make *Rice koji*, which supply various hydrolytic enzymes and vitamins for following fermentation by yeast. The property of *Rice koji* significantly affects to the flavor of Sake. *A. oryzae* encode about12, 000 genes on the genome and will play important role for the property of *Rice koji*. Some hydrolytic enzymes, such as amylases and proteinases, were well studied but most of other genes were left uncharacterized. Thus, we prepared 4 different types of *Rice koji* and performed proteome analysis using MALDI-TOF/TOF MS. As the result, 159 genes were identified as *Rice koji* protein encoding (RKP) genes. The 38 genes were well characterized and 51 genes were predicted as heat shock, secretion and primary metabolism related genes. The remained 70 genes were poorly annotated proteins. Among 159 genes, we select 85 RKP genes and disrupted using *adeA* as a marker. We success the disruption of 73 RKP genes but only heterokaryons were isolated for remained 12 RKP genes, despite of several trials. To evaluate the phenotype of RKP disruptants, we examined growth and conidia formation on plate culture. Only 2 RKP disruptants reduced their growth significantly but 11 RKPs disruptants were altered in conidia formation. Interestingly, 10 strains extremely reduced the growth in liquid culture, even though same medium was used. We further prepared *Rice koji* and evaluate the growth and enzyme production. Comparing with the plate culture, 11 RKP disruptants reduced their growth. The growth and protein production was well correlated. In this study, we could identify new genes which affect the growth end enzyme production. However, many RKP disruptants did not show any phenotypes. To examine the function of these non-phenotype RKP genes, we will prepare Sake to examine the effect to Sake metabolites and its quality.

20. Involvement of C7-C8 loop of *Aspergillus oryzae* hydrophobin RolA in interaction between RolA and a polyester. Takumi Tanaka[1], Yoonkyung Kim[1], Hiroki Tanabe[1], Kenji Uehara[1], Keiko Orui[1], Toru Takahashi[2], Keietsu Abe[1,2]. 1) Grad. Sci. Agric. Sci., Tohoku university, Sendai, Miyagi, Japan; 2) NICHe, Tohoku university, Sendai, Miyagi, Japan.

Hydrophobins are amphipathic secretory proteins with eight conserved cysteine residues and are ubiquitous among filamentous fungi. The Cys3-Cys4 and Cys7-Cys8 loops of hydrophobins are thought to form hydrophobic segments involved in adsorption of hydrophobins on hydrophobic surfaces. When the fungus *Aspergillus oryzae* is grown in a liquid medium containing the polyester polybutylene

succinate-co-adipate (PBSA), *Aspergillus oryzae* produces hydrophobin RolA, which attaches to PBSA. Here, we analyzed the kinetics of RolA adsorption on PBSA by using a PBSA pull-down assay and a quartz crystal microbalance (QCM) with PBSA-coated electrodes. We constructed RolA mutants in which hydrophobic amino acid residues in the two loops were replaced with serine, and we examined the kinetics of mutant adsorption on PBSA. QCM analysis revealed that mutants with replacements in the Cys7-Cys8 loop had lower affinity than wild-type RolA for PBSA, suggesting that this loop is involved in RolA adsorption on PBSA. In further works, we are constructing RolA mutants in which charged amino acid residues in the Cys7-Cys8 loop were replaced with alanine, and examining the kinetics of mutant adsorption on PBSA.

21. D73 of *Aspergillus oryzae* cutinase CutL1 is cooperatively involved in the ionic interaction between fungal hydrophobin RolA and CutL1 with other acidic amino acid residues of CutL1. Y. Kim[1], Y. Terauchi[2], T. Tanaka[1], Y. Tsushima[1], K. Uehara[1], T. Takahashi[3], K. Abe[1,2,3]. 1) Grad. Sci. Agric. Sci., Tohoku university, Sendai, Miyagi, Japan; 2) Dept. Agric., Tohoku university, Sendai, Miyagi, Japan; 3) NICHe., Tohoku university, Sendai, Miyagi, Japan.

Hydrophobin RolA and polyesterase CutL1 are co-expressed when the fungus *Aspergillus oryzae* is grown in a liquid medium containing the biodegradable polyester polybutylene succinate-*co*-adipate (PBSA) as the sole carbon source. RolA which adheres to PBSA interacts with CutL1 and promotes the PBSA degradation by concentrating CutL1 on the PBSA surface. In our previous studies, we revealed that positively charged amino acid residues (H32, K34) of RolA and negatively charged amino acid residues (E31, D142, D171) of CutL1 are cooperatively play an important role in the ionic interaction between RolA and CutL1. However, the amount of a CutL1 variant (E31S/D142S/D171S) recruited by a RolA variant (H32S/K34S) in the presence of NaCl (250 mM) was decreased significantly compared to that without NaCl. This result suggested some remaining charged resides in CutL1-E31S/D142S/D171S are participated in the ionic interaction with RolA.

In the present study, in order to elucidate negatively charged residues involved in RolA-CutL1 interaction other than the three residues (E31, D142, and D171) of CutL1, we selected several candidates that putatively participate in the interaction based on the alignment analysis among CutL1 homologues and analysis using 3D-structure of CutL1. Among the candidates in CutL1, we chose D73 and constructed CutL1 variants of which D73 was substituted with serine. We performed kinetic analysis of interaction between CutL1-D73S and CutL1-E31S/D73S/D142S/D171S with wild-type RolA by using Quartz Crystal Microbalance and pull-down assay with RolA-coated Teflon. The D73S substitution of CutL1 showed a decreased affinity to RolA, suggesting that D73 also cooperatively participates in the ionic interaction with RolA by the multivalent effect with other negatively charged residues of CutL1 (E31, D142, D171).

22. Functional analysis of intracellular metalloproteases, saccharolysin orthologues in *Aspergillus oryzae*. Y. Yamagata[1], Y. Ishikawa[1], H. Maeda[1], K-I. Kusumoto[2], H. Amano[3], H. Ishida[4], M. Takeuchi[1]. 1) Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Tokyo, Japan; 2) NFRI, Ibaraki, Japan; 3) Amano Enzyme Inc., Gifu, Japan; 4) Research Institute, Gekkeikan Sake Company Ltd., Kyoto, Japan.

The genome project of *A. oryzae* clarified that there were 134 genes coding proteolytic enzymes. Three genes (*olsA*, *olsB* and *olsC*) of them were estimated as orthologues of the saccharolysin gene of *S. cerevisiae*. Saccharolysin is a metalloprotease, which digests peptides produced by mitochondrial protein degradation. *S. cerevisiae* has only one saccharolysin gene, but three orthologue genes were found in *A. oryzae* genome. Thus, we presumed that the three enzymes should share biological roles in *A. oryzae*.

Deletion strain of *olsA*, *B* and *C* in *A. oryzae* (*ΔolsA*, *ΔolsB* and *ΔolsC*) showed increment of colony diameters, promotion of aerial mycelia growth and decrease of conidia formation in the presence of a mitochondrial uncoupling agent, 2, 4-dinitrophenol (DNP). Furthermore, transcription analysis on the *olsA*, *B* and *C* of wild-type *A. oryzae* in the presence of DNP revealed the transcriptions of the genes were increased in comparison with absence of DNP. Especially, *olsC* transcription level went up to more than 10 times. The results suggested OlsC should take some important roles under the mitochondrial membrane potential disappearance condition. Then overexpression strains of *olsA*, *B* and *C* were constructed. Only *olsA* overexpression strain showed decrease of conidia formation and colony diameter. The *olsA* transcription was not observed in *ΔolsC* strain, and the *olsC* overexpression caused the increment of *olsA* transcription. On the contrary, the transcription level of *olsC* was decreased by *olsA* overexpression and increased by *olsA* deletion. We considered OlsC should regulate olsA transcription and OlsA overexpression might cause feedback control against *olsC* transcription directly or indirectly. The *olsA* and *olsC* transcription should be mutually dependent, and the quantity of OlsA should be regulated strictly. This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

23. Plant biomass degrading potential of a new *Penicillium* species, *Penicillium subrubescens*. Miia R. Mäkelä[1], Sadegh Mansouri[1], Ad Wiebenga[2], Ronald P. de Vries[2], Kristiina Hilden[1]. 1) Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, Viikki Biocenter 1, University of Helsinki, Finland; 2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

A recently identified *Penicillium* species, *P. subrubescens* (Mansouri et al. 2013), was evaluated for its ability to hydrolyse plant biomass. Growth on various plant biomass related substrates demonstrated the capacity of this species to degrade all the main polysaccharides present in plant biomass as well as metabolise all their monomeric components. The ability to degrade broad range of carbohydrates suggests a high potential in plant biomass saccharification.

To evaluate this in more detail, *P. subrubescens* was grown on wheat bran and sugar beet pulp and a set of extracellular enzyme activities were analyzed from culture liquids. Also the ability to saccharify wheat bran and sugar beet pulp was determined. Compared to *P. chrysogenum*, *P. subrubescens* produced higher levels of β-glucosidase, endoglucanase, endoxylanase and cellobiohydrolase.

Enzyme mixtures produced on wheat bran by *P. subrubescens* were more efficient in saccharification of wheat bran compared to enzymes produced on sugar beet pulp cultures. The opposite result was observed for saccharification of sugar beet pulp. This demonstrates that *P. subrubescens* produces enzyme mixtures that are closely tailored to the available substrate, suggesting the presence of a fine-tuned regulatory system that controls the production of these enzymes.

Reference: Mansouri S, Houbraken J, Samson RA, Frisvad JC, Christensen M, Tuthill DE, Koutaniemi S, Hatakka A, Lankinen P (2013) *Penicillium subrubescens*, a new species efficiently producing inulinase. Antonie Van Leeuwenhoek 103:1343-1357.

24. Heterologous expression of feruloyl esterases of *Aspergillus clavatus* and *Aspergillus terreus*. Miia Mäkelä, Salla Koskela, Kristiina Hildén. Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

In the cell walls of gramineous plants, hemicelluloses are crosslinked to the aromatic lignin polymer via hydroxycinnamic acids (ferulic acid and *p*-coumaric acid). Feruloyl esterases (ferulic acid esterases, EC 3.1.1.73), classified in CAZy family CE1 (www.cazy.org), are enzymes that catalyse the cleavage of covalent ester bonds between carbohydrate and lignin moieties in plant cell walls. Due to the ability to specifically cleave ester linkages, feruloyl esterases are promising biocatalysts for a broad range of biotechnological applications. These include e.g. pharmaceutical, agricultural and food industries, as well as the production of biofuel.

Aspergillus species are one of the best studied fungi, largely due to their applicability in biotechnology and relevance in human health. In this study, putative feruloyl esterase encoding genes from *Aspergillus clavatus* and *Aspergillus terreus* were cloned and expressed heterologously in the methylotrophic yeast *Pichia pastoris*. Biochemical properties, including substrate specificity and thermotolerance of the recombinant *A. clavatus* and *A. terreus* feruloyl esterases will be presented. In addition, the ability of the recombinant feruloyl esterases to hydrolyze different plant biomass based substrates will be evaluated.

25. Forward Genetics in the white-rot fungus *Pleurotus ostreatus*: Towards identification of molecular mechanisms essential for ligninolytic system. T. Nakazawa[1], A. Izuno[1], Y. Miyazaki[2], Y. Isagi[1], Y. Honda[1]. 1) Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto, Japan; 2) Forestry and Forest Products Research Institute, Tsukuba, Japan.

Lignin, one of the major woody biomass, is a complex and irregular aromatic polymer mainly consisting of phenylpropanoids, which makes it very highly resistant to biodegradation by microorganisms in nature. The white-rot fungus (basidiomycete) plays an important role in the global carbon cycle: Lignin is degraded (depolymerization) exclusively by the white-rot fungus. Peroxidases, lignin peroxidases (LiP), manganese peroxidases (MnP) and versatile peroxidases (VP), have been reported to be involved in lignin degradation (depolymerization). However, there has been also suggested to be factors other than the “ligninolytic enzymes” themselves that play an important role in lignin degradation . We here present a forward genetics study in the white-rot fungus *Pleurotus ostreatus*, the oyster mushroom, to reveal molecular mechanism(s) essential for the ligninolytic system. For a preliminary study, we first introduce mutations to strain PC9 through a UV irradiation, resulting in isolation of four mutants defective in decolorization of Orange II, which has been reported to be a substrate specific for MnP in *P. ostreatus*. As is expected, Mn^{2+}-dependent and -independent oxidation activities of guaiacol are not detected in all of the mutants. F_1 analysis suggests that mutations in a single gene cause the mutant phenotype in all of the four mutants, respectively. We then construct new genetic markers between PC9 and PC15 to identify the genomic region close to the mutated gene. We successfully identify markers linked to the mutated phenotype in three of the four mutants. Resequencings of genomes of the mutants let us find mutated genes close to the linked genetic markers. Now, we are trying making it clear that they are essential for decolorizeation of Orange II and secretory production of active peroxidases in *P. ostreatus* through a complementation test (for a recessive phenotype) or an introducing the mutated gene (for a dominant phenotype).

26. Colocalization of wood modifications and secretome composition during the colonization of wood by *Postia placenta* and *Gloeophyllum trabeum*. Gerald Presley, Jiwei Zhang, Jonathan Schilling. Bioproducts and Biosystems Engineering, University of Minnesota, 2004 Folwell St. Paul, MN 55108.

Wood decay fungi drive the mineralization of the largest biotic sink of carbon on earth, but the biochemistry behind this process is not fully understood. Brown rot fungi are theorized to generate an extracellular fenton system to generate hydroxyl radicals that degrade wood polysaccharides. They also secrete hydrolytic enzymes which may be spatially separated from the fenton system. Attempts to recreate brown rot *in vitro* have not been successful and thus the mechanistic paradigm is incomplete. To address this we have developed a wood wafer decay system that utilizes a spatial gradient along an advancing hyphal front to co-localize wood modifications and secretome variations along a fine-scale time series of decay stages. Two model brown rot fungi, *Gloeophyllum trabeum* and *Postia placenta* were grown up spruce wafers and total extracellular protein was extracted from 5 mm segments along the advancing hyphal front. Screening for hydrolytic enzyme activity showed that endo-acting hydrolase (cellulase, mannanase, and xylanase) specific activity was only detectable 5 mm or farther behind the advancing hyphal front and it increased thereafter reaching a plateau in the most decayed portions of the wafer. Identification of proteins from the same extracts with LC-MS showed differential expression of proteins along the advancing hyphal front. In early decay stages, less than 5 mm behind the hyphal front, peptides from low molecular weight proteins of unknown function along with some hemicellulose-degrading glycosyl hydrolases were by far the most abundant. In later stages of decay, endo-acting glycosyl hydrolases including the major known endocellulases from *P. placenta* and *G. trabeum* were the most commonly identified proteins. The colonization and decay of wood is a dynamic process as shown by the changes in the secretome from early to late decay stages.

27. *Debaryomyces hansenii* killer toxin against Candida species. Nabaraj Banjara, Hallen-Adams Heather. Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE.

Candida yeasts are commensal members of the gastrointestinal, mucosal, oral and vaginal microbiota. However, when the host defense

system and microbiota are disturbed, *Candida* can become pathogenic and cause severe infection (candidiasis). Antifungal drugs targeted to treat candidiasis have been shown to result in treatment failures due to development of resistance during long term antifungal therapy. *Debaryomyces hansenii* is the most common yeast species found in cheeses. Some strains of *D. hansenii* produce killer toxins - toxic proteins or glycoproteins which can kill sensitive yeast species. Therefore, we investigated whether *D. hansenii* isolated from different cheese samples had an inhibitory role on *Candida* species. Forty two *D. hansenii* isolates were collected from different types of cheese and killer activity against *Candida albicans* and *Candida tropicalis* was screened by the streak-plate agar diffusion bioassay. Killer activity among *D. hansenii* strains at different pH values (4.5, 5.0, 5.5, 6.0) and temperatures (20 C, 25 C, 30 C and 35 C) was quantified by agar diffusion well bioassay; the effect of *D. hansenii* killer toxin on *C. albicans* and *C. tropicalis* growth kinetics was also studied. Twenty three strains (54%) of *D. hansenii* demonstrated killer activity against *C. albicans* and *C. tropicalis* and killer toxin activity differed among the *D. hansenii* strains. *D. hansenii* killer toxin was active against *C. albicans* up to pH 5.5 but against *C. tropicalis* to pH 6.0. Killer activity was higher at low temperature and low pH. Killer toxin activity was detected up to 35 C against *C. albicans;* for *C. tropicalis*, lower temperatures were required to observe a killer effect. Incubation of killer toxins from three strains Dhans-237, Dhans-274 and Dhans-65 at 25 C decreased killer activity by 3%, 15 % and 11% after 24 hours and 11%, 20% and 29% after 48 hours, respectively. In contrast, we observed killer activity for short time period when toxins are incubated for 37 C. The results confirmed that same killer toxin from *D. hansenii* can act differently in different species, temperature and pH conditions; strains such as Dhans-237, which have activity at higher temperature, may have medical application.

28. Force-Sensing Amyloids in Yeast Adhesins Mediate Adhesion and Biofilm Formation. cho xj chan[1], peter n lipke[2], melissa garcia-sherman[2], desmond jackson[2], ivor joseph[2]. 1) pace university, new york, NY; 2) brooklyn college, brookyn, NY.

The *Candida albicans* adhesin Als5p has an amyloid-forming sequence that is required for aggregation and formation of model biofilms on polystyrene (Alsteens et al., 2010 PNAS 107:20744; Garcia et al., PLoS One 6:e17632). Similarly, *Saccharomyces cerevisiae* Flo11p and Flo1p adhesins have amyloid-forming sequences (Ramsook et al., 2010 Eukaryot. Cell 9:393-404). These amyloid sequences can form high avidity surface arrays of adhesins called nanodomains. Because amyloid formation can be triggered by force, we investigate whether mechanical turbulent flow from vortex mixing and laminar shear flow could induce formation of amyloid nanodomains. We test whether amyloid formation from force could increase cellular binding to surfaces and biofilm formation. Vortex-mixing cells expressing $Als5p^{WT}$ for 60 seconds increased aggregation and adhesion of cells to BSA-coated beads 1.7-fold compared to cells that were not vortex-mixed. There was little shear-activated increase in adhesion or thioflavin fluorescence in cells expressing an amyloid-impaired V326N substituted Als5p. Shear from laminar flow at 0.8 dynes/cm^2 increased quantity and strength of cell-to-surface and cell-to-cell binding, compared to shearing at 0.02 dynes/cm^2. Shear-induced binding led to formation of robust biofilms. Thioflavin T fluorescence showed that the vortex-mixing and laminar flow also induced surface amyloid nanodomains in Als5p-expressing cells. Inhibitory concentrations of amyloid-dyes thioflavin S, Congo red, or a sequence-specific anti-amyloid peptide decreased activation of adhesion. Similarly, *S. cerevisiae* cells expressing Flo1p and Flo11p flocculins showed 2.0-fold increases in the flocculation rate following vortex-mixing. Flocculin-expressing cells also showed shear-dependent binding, amyloid formation, biofilm formation, and inhibition by anti-amyloid compounds. The effects of vortex-mixing were replicated in heat-killed cells as well. Together these results show that shear force exerted by laminar or turbulent flow leads to formation of amyloid nanodomain on the surface of cells, which in turn mediates aggregation of yeast cells, adhesion to surfaces, and biofilm formation.

29. Phenotypic switching in *Candida albicans*: towards integrating environmental inputs and cellular outputs. Iuliana Ene, Richard Bennett. Molecular Microbiology and Immunology, Brown University, Providence, RI.

The ability to switch between different phenotypic states is crucial for the success of *Candida albicans* as a human pathogen. While the white-opaque switch modulates mating, the yeast-hyphal transition (filamentation) promotes invasion and damage to host tissues. These transitions are associated with different mammalian niches or stages of infection, yet the molecular basis of their preference for these niches is poorly understood. To understand the metabolic differences between phenotypic states, we carried out a comparative analysis of white and opaque cells using Phenotype MicroArrays. This high-throughput analysis allowed monitoring of growth, white-opaque switching and filamentation of *C. albicans* cells under ~2,000 different nutrients or stressors. Integrating the phenotypic data with the annotated genome and the metabolic network of *C. albicans* allowed us to identify metabolic pathways and regulatory processes that are co-regulated with phenotypic transitions. We found that >80% of conditions promoted the growth of white cells to a higher biomass than that of opaques, and that opaque cells were more sensitive than whites to most stressors tested. The increased fitness of white cells was more apparent at 37°C than at 25°C. As expected, switching from white to opaque was favoured by growth at lower temperatures, although several conditions induced this switch at 37°C. We also identified a number of conditions that stabilized the opaque state at 37°C, suggestive of niches where these cells might be stable in the host. Carbon sources were important regulators of the white-opaque switch and subsequent genetic analyses identified glucose sensing and regulatory pathways as key modulators of opaque stability. In parallel, we identified novel nutritional cues that induce the program of filamentation, as well as significant overlaps between the regulation of the white-opaque switch and the yeast-hyphal transition. Our findings underline key differences in the metabolic programs of *C. albicans* cells in alternate phenotypic states. This will allow identification of host niches that promote these phenotypic transitions and understanding of the role of metabolic adaptation in the lifestyle of *C. albicans*..

30. Translation stress signaling to the circadian clock of *Neurospora crassa*. Axel Diernfellner, Linda Lauinger, Michael Brunner. AG Brunner, Biochem Ctr Heidelberg, Heidelberg, Germany.

The *Neurospora crassa* clock protein FREQUENCY (FRQ) is the negative regulator of the circadian transcription factor WHITE COLLAR COMPLEX (WCC). In the course of a day, FRQ is progressively phosphorylated at more than 100 sites - mainly mediated by its interaction partner CASEIN KINASE 1a (CK1a) - and eventually inactivated as a repressor and degraded. Thus, timed phosphorylation of FRQ is crucial for circadian timekeeping. However, translation inhibition causes rapid hyperphosphorylation of FRQ in a non-circadian

fashion. We show that this hyperphosphorylation is mediated by the *Neurospora* homolog of checkpoint kinase 2.

31. Functional analysis of secondary metabolism gene clusters expressed in *Colletotrichum* appressoria. Jean-Felix Dallery[1], Antonios Zampounis[1], Emilie Adelin[2], Sandrine Pigne[1], Olivier Lespinet[3], Jamal Ouazzani[2], Marc-Henri Lebrun[1], Richard O'Connell[1]. 1) UMR1290 BIOGER-CPP, INRA-AgroParisTech, Avenue Lucien Bretignieres, 78850 Thiverval-Grignon, France; 2) Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles ICSN, Centre National de la Recherche Scientifique, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France; 3) Institut de Genetique et Microbiologie, UMR8621 CNRS-Universite Paris-Sud, UFR des Sciences, 91405 Orsay cedex, France.

Species of the genus *Colletotrichum* cause devastating anthracnose or blight diseases on numerous crop plants worldwide. *C. higginsianum* uses a hemibiotrophic strategy to infect *Arabidopsis* and other Brassicaceae. Compared to other hemibiotrophs, its genome contains an exceptionally large number of genes (78) encoding secondary metabolism (SM) key enzymes. As in other fungi, these key genes are organized into clusters that also contain genes encoding accessory enzymes of the same biosynthetic pathway and usually efflux transporters and pathway-specific transcription factors. A remarkable finding from RNA-Seq transcriptome profiling was that 27 SM clusters are specifically expressed only *in planta* by appressoria and/or biotrophic hyphae. Since each cluster potentially synthesizes one final metabolite, this suggests appressoria and biotrophic hyphae deliver a cocktail of different metabolites to the first infected host cell. At this early stage host cells remain alive, raising the possibility that these molecules function similar to protein effectors in host manipulation, rather than as simple phytotoxins. Work is ongoing to decipher how the expression of these SM gene clusters is so precisely regulated during the course of infection. We targeted *C. higginsianum* orthologs of the well-characterized global SM regulators *LaeA*, *Hp1*, *Dim5*, *CclA* and *Kmt6* for genetic manipulation, with a view to inducing the fungus to synthesize appressorial metabolites in large quantities in culture. The aim is to obtain sufficient material for identification and structural determination (mass spectrometry, NMR) and for functional assays to evaluate their biological activities against plants, bacteria and fungi.

32. The proteomic analyzes of *Paracoccidioides lutizii* conidia by using MS^E approach. Andre Moreira[1], Vanessa Cruz-Leite[1], Alexandre Bailão[1], Juliana Parente[1], Celia Soares[1], Orville Ruiz[3], Ana Flavia Parente[2], Clayton Borges[1]. 1) Federal University of Goias,Goiania, Goias,Brazil; 2) Federal University of Amazonas,Manaus, Brazil; 3) Corporación para Investigaciones Biológicas,Medellín,Colombia.

Paracoccidioidomycosis (PCM) is an endemic mycosis in Latin America. The PCM is a systemic disease caused by the thermo dimorphic fungus *Paracoccidioides* spp, which can exist as mycelia and yeast forms. The mycelia form can be found in environment at temperatures between 18 °C and 25 °C and produces spores or conidia. The PCM infection route occurs by the inhalation of conidia. Once in the lungs, these particles are converted into yeasts pathogenic form. Until now, metabolic aspects and virulence factors related to *Paracoccidioides lutizii* infective propagule are little understood. In this concern, a global proteomic study of *P. lutizii* conidia was performed. For conidia production, the mycelia of the isolate *Pb*01 were cultured in potato agar medium during 90 days at 18 °C. Obtained conidia were collected and purified. The proteins were extracted and subjected to tryptic digestion followed by identification by *NanoUPLC-MS^E* approach. We identified a total of 242 proteins in *P. lutizii* conidia which were subjected to *in silico* functional analysis Proteins putatively acting as adhesins, such as GAPDH, enolase, and the glycoprotein β-1,3-glucanosyltransferase (gel2) were identified during our analysis. We also identified proteins related to signal transduction pathways, such as Ras and RhoA GTPases, previously demonstrated to be required for morphologic changes in pathogenic fungi. Proteins related to evasion, defense and virulence were also identified. Proteins acting during temperature shifts or oxidative stress provided by the host environment such as HSP90, catalase, mitochondrial peroxiredoxin Prx1 were identified. It were also identified proteins related to energy production and protein biosynthesis, which can provide important aspects to survival of this resting cells. These results highlight that *P. lutizii* conidia contain proteins that can contribute to its maintenance in the environment and also molecules related to host-pathogen interactions.

33. Oxylipins in *Fusarium verticillioides* and Fumonisin accumulation in Maize. Valeria Scala[1], Chiara Dall'Asta[2], Paola Giorni[3], Francesca Cardinale[4], Ivan Visentin[4], Martina Cirlini[2], Matteo Ludovici[1], Paola Battilani[3], Massimo Reverberi[1], Corrado Fanelli[1]. 1) Department of Environmental Biology, Sapienza University of Roma, Roma, Rm, Italy; 2) Department of Food Science, University of Parma, Pr, Italy; 3) Istitute of Entomology and Plant Pathology, University Cattolica del Sacro Cuore, Piacenza, Pc, Italy;; 4) Department of Agriculture, Forestry and Food Science, University of Turin, To, Italy.

Fusarium verticillioides gained centre stage for maize cropping in 1989, when the mycotoxins fumonisins were discovered. The involvement of fatty acids, both in hybrid susceptibility to contamination and in fumonisin accumulation and masking were also described recently. Therefore, oxylipins and their role in host plant-fungus cross talk and in *F. verticillioides* physiology were considered, and studied following a multifaceted approach. We used a combination of biological (fungal characterization, virulence quantification, mutant generation), chemical (fumonisin quantification, lipidomic analysis) and molecular (fungal and plant gene expression, ChIP analysis) approaches to study the different roles of oxylipins in shaping *F. verticillioides* behaviour. The results confirm the role of oxylipins in fungal physiology, growth and ability to turn on secondary metabolism. The involvement of lipids signals in fungal infection of maize and toxin accumulation was confirmed. Four lipid entities differentiated highly- from poorly-contaminated samples (cut-off of 2000 µg of fumonisins/Kg kernels), confirming that sphingolipid and oxylipin metabolism interfere with *F. verticillioides* fumonisin production in maize kernel in open field.

34. Real-time imaging of hydrogen-peroxide dynamics in vegetative and invasive hyphae of *Fusarium graminearum* using the novel fluorescent reporter HyPer. Michael Mentges, Joerg Bormann. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

Balanced dynamics of reactive oxygen species in the phytopathogenic fungus *Fusarium graminearum* play key roles for development and infection. To monitor those dynamics, ratiometric analysis using the novel, hydrogen-peroxide (H_2O_2) sensitive fluorescent indicator protein HyPer-2[1] was established for the first time in a filamentous fungus. H_2O_2 abundance changes the excitation spectrum of HyPer-2

with an excitation maximum at 405 nm for the reduced and 488 nm for the oxidized state, facilitating ratiometric readouts with a maximum emission at 516 nm. HyPer-2 analyses were performed using a microtitre fluorometer and confocal laser scanning microscopy (CLSM). Addition of external H_2O_2 to mycelia caused a steep and transient increase in fluorescence excited at 488 nm. This can be reversed by the addition of the reducing agent DTT. HyPer-2 in *F. graminearum* is highly sensitive and specific to H_2O_2 even in tiny amounts. Hyperosmotic treatment and elevation of temperature elicited a transient internal H_2O_2 burst. Hence, HyPer-2 is suitable to monitor the intracellular redox balance. Using CLSM, developmental processes like septation, nuclear division, tip growth and infection structure development were analyzed. Yet, none of these processes implied marked fluctuations in intracellular H_2O_2. To test the significance of H_2O_2 produced by the NADPH oxidase complex, the regulatory subunit of the NOX complex, NOXR, was knocked out in a HyPer-2-expressing strain. Surprisingly, those mutants displayed a relatively higher steady state oxidation level when compared with the parental strain. Similar analyses with a deletion mutant of the putative regulator of the oxidative stress response Fgap1 showed no differences to the HyPer-2 expressing wild-type derived strain.

Taken together, HyPer-2 is a valuable and reliable tool for the characterization of mutants and the assessment of environmental conditions.

[1] Markvicheva KN, et al. (2011). Bioorg Med Chem 19: 1079-1084.

35. Identification and characterization of a NADPH oxidase target in *Fusarium graminearum*. Salima Chatur[1,2], Manisha Joshi[1], Chris Rampitsch[3], Li Wang[1], Gopal Subramaniam[1]. 1) AAFC 960 Carling Avenue Ottawa, ON Canada K1A 0C6; 2) Carleton University Department of Biology 1125 Colonel By Drive Ottawa, ON Canada K1S 5B6; 3) AAFC 101 Route 100 Morden, MB Canada R6M 1Y5.

F. graminearum is a fungal plant pathogen that causes Fusarium Head Blight (FHB) on important food and feed cereal crops including wheat, maize and barley. It is ranked as a major global plant pathogen causing significant yield reduction resulting in economic losses. Earlier study identified distinct roles for NADPH oxidase (NOX) genes in *F. graminearum.* NOX enzymes generate reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), which are important in signal transduction. The study indicated that while Δ*noxA* strain has reduced superoxide production and is unable to develop sexual fruiting bodies and ascospores, the Δ*noxA*/*B* double mutant has reduced pathogenicity on wheat. To elucidate the mechanism of NOX enzymes, an LC-MS approach was used to examine redox changes in the *ΔnoxA/B* proteome compared to wild-type *F. graminearum* strain. Samples were enriched for redox sensitive cysteine residue(s) and candidate proteins were identified via shotgun (gel free) linear ion trap mass spectrometery. Deletion analyses and overexpression of one of the candidate genes with modified cysteine residues confirmed that it is likely a genuine substrate of the NOX enzyme complex. Deletion of *Fg10089* as well as modification of this cysteine residue results in reduced virulence on wheat. However, no differences in the production of 15-A deoxynivalenol in culture is observed. The further characterization of this protein and its contribution to *F.graminearum* physiology and function will be examined.

36. Biosynthesis of DON/15-ADON and NX-2 by different variants of *TRI1* from *Fusarium graminearum*. Gerlinde Wiesenberger[1], Elisabeth Varga[2], Philipp Fruhmann[3], Romana Stückler[1], Todd Ward[4], Rudolf Krska[2], Franz Berthiller[2], Gerhard Adam[1]. 1) Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU), 3430 Tulln, Austria; 2) BOKU, Department for Agrobiotechnology (IFA-Tulln), Center for Analytical Chemistry and Christian Doppler Laboratory for Mycotoxin Metabolism, 3430 Tulln, Austria; 3) Institute of Applied Synthetic Chemistry, Vienna University of Technology, 1060 Vienna, Austria; 4) USDA-ARS, Bacterial Foodborne Pathogens & Mycology Research Unit, PEORIA, IL, 61604-3999, USA.

Fusarium graminearum is one of the economically most important plant pathogens causing diseases such as Fusarium Head Blight (FHB) of small grain cereals and ear rot of maize. During a large scale survey of *F. graminearum* (*sensu strictu*) in the northern US strains (N-strains) had been discovered (Liang *et al*., 2014), which show normal aggressiveness and produce a novel type A trichothecene termed NX-2. This mycotoxin looks like 3-ADON, but lacks the keto group at C-8 (Varga *et al*., 2014).

In various *Fusarium* species oxidation at C-7 and C-8 of trichothecenes is performed by P450 monooxygenases encoded by *TRI1* genes. We sequenced the *TRI1* genes of several N-isolates and found 14 AA changes between the Tri1 proteins of PH-1 and the N-strains. By swapping the coding regions of the DON/15-ADON producing strain PH-1 and one of our N-isolates (WG-9) we confirmed, that the N-variant of the Tri1 protein is responsible for the specific oxidation at the C-7 of NX-2 (Varga *et al*., 2014).

We constructed hybrid genes to investigate which amino acids in the Tri1 proteins account for the specific oxidation reactions. Since functional tests are tedious and time consuming in *Fusarium*, we have set up a yeast system for investigation of the Tri1 proteins. We purified large amounts of calonectrin, which is an intermediate compound and the substrate for the Tri1 monooxygenase and plan to feed this to a newly constructed trichothecene resistant *Saccharomyces cerevisiae* strain expressing the *TRI1* cDNAs from PH-1, WG-9 and various hybrid genes.

37. Alteration of ergot alkaloid profile in *Epichloë coenophiala* by surgical trim of a chromosome end. Simona Florea[1], Mark L. Farman[1], Daniel G. Panaccione[2], Christopher L. Schardl[1]. 1) Plant Pathology, University of Kentucky, Lexington, KY; 2) Division of Plant & Soil Sciences, West Virginia University, Morgantown, WV.

Tall fescue (*Lolium arundinaceum*) is an agronomical important cool season grass inhabited by *Epichloë coenophiala*, a fungal symbiont, whose presence greatly increases the plant resistance to different biotic and abiotic stresses. Strains of *E. coenophiala* can produce a complex cocktail of toxic compounds such as ergot alkaloids, which are known to be the culprits for fescue toxicosis on grazing animals. These compounds pose major risks to livestock health and detrimental effect to their performance, with annual US losses of ca. $1 billion yearly. *Epichloë coenophiala* e19 strain is a hybrid of three parental species, two of which contributed homologous ergot alkaloid gene

clusters, designated *EAS*1 and *EAS*1. The genome sequencing of this strain revealed that the two ergot alkaloid gene clusters are telomeric. This information prompted us to develop a strategy that trimmed in a non-transgenic manner the chromosome ends harboring the ergot alkaloid cluster. Through this technology, we developed fungal strains that would confer the crucial benefits to the grass but would be free of toxic ergot alkaloid ergovaline. Such strains introduced into elite tall fescue cultivars would have superior performance over the existing commercial cultivars due to improvements in both grass and its endophyte.

38. *trp1*$^+$ vector transformation in auxotrophic *Coprinopsis cinerea* strains generates less clones than co-transformation with a non-*trp1*$^+$ vector. B. Dörnte, U. Kües. Dept Molec Wood Biotech, Univ Goettingen, Goettingen, Germany.

Genetic transformation of *Coprinopsis cinerea* as first described by Binninger et al. (1987) makes use of the integrative vector pCc1001 with the *C. cinerea* tryptophan synthetase gene *trp1*$^+$. This gene allows the complementation of *trp1*$^-$ auxotrophies in the fungus and serves as a selection marker in protoplast transformations. During such process, the transforming DNA integrates, singly or in tandem, at one or more ectopic sites into the host genome. Multiple integrations offer the chance of co-transformation of different vectors at the same time. With equal amounts of vectors, equal numbers of nuclear sites should then become occupied by two distinct DNAs. Using pCc1001 for transformation revealed however a surprising phenomenon. Co-transformation with a non-*trp1*$^+$ vector doubles numbers of transformants compared to the single *trp1*$^+$ vector transformation. In contrast, using the selection marker *pab1*$^+$ (for PABA synthetase) resulted in single and in co-transformation in the same numbers of transformants. Thus, the phenomenon is specific to *trp1*$^+$. Neither lengths modification of the *trp1*$^+$ harboring fragment in pCc1001 nor changing the vector backbone eliminated the phenomenon. Testing different relative *trp1*$^+$ vector to non-*trp1*$^+$ vector concentrations showed that a decreasing concentration of the non-*trp1*$^+$ vector in co-transformations reduces the number of clones, finally down to the level of the single vector transformation. These results lead to the conclusion that multiple integration of *trp1*$^+$ copies in single-transformation may lead to Trp1 and so to tryptophan overproduction and, in consequence, to a shutdown of the tryptophan biosynthesis pathway due to feedback inhibition. Higher numbers of clones in co-transformations might then be explained by block of potential insertion sites for the *trp1*$^+$ vector by the non-*trp1*$^+$ vector DNA. For verification, we tested the effect of site specific DNA integration in a non-homologous-end-joining deficient strain and the above phenomenon disappeared. Also in line with this conclusion, addition of the tryptophan precursors (anthranilate, indole) or the aromatic amino acids (phenylalanine, tyrosine) strongly reduced the numbers of *trp1*$^+$ clones in single and co-transformations.

39. Switching the fate of mRNA transcripts for mitochondrial biogenesis in *Saccharomyces cerevisiae*. Chien-Der Lee, Benjamin Tu. UTSW, Dallas, TX.

PUF proteins are conserved post-transcriptional regulators that bind in a sequence-specific manner to the 3'UTRs of mRNA transcripts. Paradoxically, PUF proteins have been proposed to promote both degradation of their target mRNAs as well as their translation. Herein, we show how a yeast PUF protein Puf3p responds to glucose availability to switch the fate of its bound transcripts that encode proteins required for mitochondrial biogenesis. Upon glucose depletion, Puf3p becomes phosphorylated, associates with polysomes, and promotes translation of its target mRNAs. As such, nutrient-responsive phosphorylation toggles the activity of Puf3p to promote either degradation or translation of these mRNAs according to the needs of the cell. Such activation of translation of pre-existing mRNAs might enable rapid adjustment to environmental changes without the need for de novo transcription. Strikingly, a Puf3p mutant that prevents its phosphorylation no longer promotes mRNA translation but also becomes trapped in intracellular foci in an mRNA-dependent manner. Our findings suggest how the inability to properly resolve Puf3p-containing mRNA-protein granules via a phosphorylation-based mechanism might be toxic to a cell.

40. Production of natural statins using heterologous expression in *Aspergillus oryzae*. Magdalena Koziol, Jeroen Maertens, Colin M. Lazarus. University of Bristol, Bristol, United Kingdom.

Statins are HMG-CoA reductase inhibitors and, as inhibitors of cholesterol biosynthesis, have been prescribed as the world's most valuable pharmaceuticals. Pharmaceutical preparations, such as simvastatin and pravastatin, are produced semi-synthetically following fermentation of producers of the natural products compactin (*Penicillium citrinum*) and lovastatin (*Aspergillus terreus*), while atorvastatin is a fully synthetic product. Heterologous production of lovastatin in *Aspergillus oryzae* could be achieved by transferring 5 genes from *A. terreus* on 2 multigene expression vectors. One vector carried genes encoding a nonaketide synthase, an enoyl reductase and a cytochrome P450, while the other vector carried genes for a diketide synthase and an acyl transferase whose function is to join the polyketide chains. However lovastatin production was enhanced by the inclusion of a sixth gene encoding a thioesterase required to release the nonaketide from the synthase enzyme. This work has been replicated using genes from a *P. citrinum* gene cluster to produce compactin in *A. oryzae*. Since lovastatin and compactin differ only by a single methyl group on the nonaketide, work is currently in progress to assess the specificities of the enzymes that have the methylated or non-methylated nonaketide chain as a substrate, and to investigate the reciprocal conversion of the nonaketide synthases for lovastatin and compactin synthesis by domain swapping.

41. Molecular characterization of secondary metabolism in the phytopathogenic fungus *Ustilago maydis*. ESMERALDA REYES FERNANDEZ[1], ZAKARIA BARIE[2], MARC STRICKERT[3], HELGE BODE[2], MICHAEL BÖLKER[1]. 1) University of Marburg, Department of Genetics, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; 2) Goethe University, Institute for Molecular Life Sciences, Max-von-Laue-Str. 9 Biocenter, N250, 60438 Frankfurt, Germany; 3) University of Marburg, Department of Mathematics and Computer Science, Hans-Meerwein-Str. 6, 35032 Marburg, Germany.

Ustilago maydis is a well established model organism for the study of plant-microbe interactions although its biosynthetic potential has not been totally explored. In this work, we focused our attention on identifying potential secondary metabolite gene clusters and their biological function in *U. maydis*. Bioinformatics approaches as SMURF (Secondary Metabolite Unknown Region Finder) and antiSMASH (antibiotics and Secondary Metabolite Analysis Shell) allowed the prediction of a gene cluster (Cluster "A") that was further verified by manual inspection. The gene cluster was composed of 14 genes, including a transcription factor, three polyketide synthases and a

cytochrome P450 monooxygenase. Overexpression of the transcription factor resulted in the activation of the whole cluster and triggered the production of a black-greenish pigment after prolonged incubation. LC-MS analysis revealed a mix of compounds derived from tetrahydroxynapthalene. We are currently generating single knockout mutants in order to have a better understanding of the role of each gene in the biosynthesis of these compounds.

42. Expression of *Agaricus bisporus* manganese peroxidases in *Schizophyllum commune*. Aurin Vos[1], Tom van den Brule[1], Klaas Nierop[2], Luis Lugones[1], Han Wösten[1]. 1) Biology, Utrecht University, Utrecht, the Netherlands; 2) Earth Sciences, Utrecht University, Utrecht, the Netherlands.

White rot fungi are thought to degrade lignin using AA2 enzymes like lignin peroxidase, manganese peroxidase (MnP) and versatile peroxidase. It is unclear if *S. commune* is a white rot or brown rot fungus. This basidiomycete is a poor lignin degrader. No genes encoding AA2 enzymes are found in its genome, while it is enriched in AA9 genes. This would place *S. commune* in between brown and white rot fungi. We here introduced two *A. bisporus mnp* genes in *S. commune*. qPCR verified expression of these genes in *S. commune*. Decoloration of RBBR by *mnp* expressing *S. commune* strains indicated production of active MnP. This was confirmed using the MBTH/DMAB assay. Addition of hemin to standing cultures increased MnP activity 5-fold. Pyrolysis analysis indicated that a *S. commune* strain expressing both MnPs of *A. bisporus* degraded lignin in birchwood . The G subunit of lignin seems to be preferentially degraded. These data show that the ligninolytic pathway of white rot fungi can be reconstituted in *S. commune*.

43. Heterologous expression of natural product biosynthesis genes in *Aspergillus oryzae*. Marie Dollmann, Pitchapa Markwalder, Hélène Lecoq, Anne Schitter, Christian Thibaut, Kathrin Buntin, Klaus Memmert, Charles Moore, Frank Petersen, Dominik Pistorius, Esther Schmitt, Tim Schuhmann, Ying Wang, Eric Weber, Joanne Wong. Natural Products Unit, Novartis Institutes for Biomedical Research, Lichtstrasse 35, CH-4002 Basel, Switzerland.

Filamentous fungi are a prolific source of bioactive compounds. The advent of rapid and economical genome sequencing has shed light on a multitude of gene clusters encoding the biosynthesis of natural products, most of which contain polyketide synthases (PKSs) or non-ribosomal peptide synthetases (NRPSs). However in most cases such gene clusters are silent, and a heterologous expression system is warranted to elucidate gene function and to facilitate the production and chemical characterization of the compounds. We will present our results in the expression of fungal megasynthases in *Aspergillus oryzae*.

44. Proteomic analysis during hyperosmotic stress revels a complex osmoadaptative repertoire by the pathogenic dimorphic fungus *Paracoccidioides* sp. Leandro Rodrigues[1], Wesley Brito[1,2,3], Ana Flávia Parente[4], Alexandre Bailão[1], Célia Soares[1]. 1) UFG - ICBII, Goiânia, GO; 2) UEG - UnuCET, Anápolis, GO; 3) UniEVANGÉLICA, Anápolis, GO; 4) UFAM - ICB, Manaus, AM, Brazil.

Paracoccidioidomycosis (PCM) is an important human systemic mycosis with a broad distribution in Latin America. The disease is caused by the dimorphic fungus *Paracoccidioides* sp. The infection occurs primarily in the lungs, from where the fungus can disseminate via the bloodstream and/or lymphatic system to all parts of the body, rendering the disseminated form of PCM, and in this way the pathogen deal with changes in the osmotic pressure. In this work, we have evaluated the proteomic profile of yeast cells of *Paracoccidioides* sp., isolate Pb01 (ATCC MYA – 826), in hyperosmotic stress condition induced with 0.1M of KCl. The increase in transcriptional levels of hog (high osmolarity glycerol) after 6 hours at high salt concentration was used to define the exposure time and the concentration to stressor agent. Also, cell viability was checked by using propidium iodide as dead cell marker. Yeast cells were subsequently submitted to protein extraction and tryptic digestion. Peptides were separated by NanoUPLC and analyzed by mass spectrometry using a label free quantification approach (MS^E). Three hundred seventy five protein species were identified as differentially expressed in the osmotic stress condition and were classified according FunCat2. The results suggest a cell wall remodeling, considering the increased expression of glucan biosynthesis enzymes and the decreased expression of chitin biosynthesis enzymes during hyperosmotic stress. This hypothesis was reinforced by the altered content of cell wall polyssacharides detected by both, cell wall carbohydrate quantification and chitin detection by fluorescence microscopy in the presence of Calcofluor White. In addition, it was also observed alterations in the energy metabolism, converging the metabolic products to glycerol synthesis. Further, proteins involved in amino acid metabolism and hydrogen peroxide detoxification reactions were modulated. Hereupon, our study suggests that *Paracoccidioides* sp. is able to minimize the effects caused by hyperosmotic stress presenting a vast osmoadaptative repertoire.

45. A nitrogen tranceptor in the oleaginous yeast *Yarrowia lipolytica*. Erin Bredeweg, Kyle Pomraning, Young-Mo Kim, Thomas Metz, Scott Baker. Cell Isolation and Systems Analysis, EMSL at PNNL, Richland, WA.

Membrane bound permease-like proteins located at the plasma membrane aid fungi in sensing nutrient molecules in the surrounding environment. Detection of molecules unleashes a cascade of signals which alter transcription and translation of relevant acting permeases. The amino acid sensor SSY1 in *Saccharomyces cerevisiae* signals downstream to a kinase cascade through PTR3, and to a self-cleaving protease SSY5, which goes on to activate transcription factors STP1 and STP2. The oleaginous yeast *Yarrowia lipolytica* contains homologues to these proteins, which feature domains relevant to their functions in *S. cerevisiae*. We are evaluating mutants of the *Y. lipolytica* SPS complex (ssy1-ptr3-ssy5) for their ability to utilize, respond to, and uptake amino acids. *Y.lipolytica* PTR3 is upregulated during later nitrogen limitation, concurrent with lipid accumulation. *Y. lipolytica* accumulates a high concentration of triglycerides in lipid droplets, a characteristic which is being developed for production of biofuel precursors. However, little work has been done on the regulation of lipid metabolism in *Y. lipolytica*, which is dependent upon carbon and nitrogen availability. Understanding nitrogen sensing and signaling in this fungus may improve it as a host for efficient biofuel production.

46. Duplication and redundancy of leucine, valine and isoleucine biosynthesis genes in *Aspergillus nidulans*. Damien Downes, Pierre Migeon, Cameron Hunter, Richard Todd. Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA.

The branched chain amino acids (BCAA) leucine, isoleucine, and valine are essential dietary amino acids in mammals; fungi, however, can synthesize these three amino acids. For this reason, BCAA biosynthesis enzymes have been suggested as possible drug targets for treatment of infections by opportunistic pathogens such as *Aspergillus fumigatus*. Synthesis of the three BCAAs has been well characterized in the yeast *Saccharomyces cerevisiae*. However, recent work on the BCAA pathway enzymes dihydroxyacid dehydratase in *A. fumigatus* and α-isopropylmalate synthase *Aspergillus nidulans* has shown divergence with *S. cerevisiae* in the number of genes encoding functional enzymes for these steps. The final two steps of leucine biosynthesis are carried out by β-isopropylmalate dehydrogenase (β-IDH) and BCAA aminotransferase (BAT), but the genes encoding these enzymes have not yet been characterized in the Aspergilli. In *S. cerevisiae*, there is one β-IDH gene and two BAT genes. The BATs also catalyze the final step of isoleucine and valine production. Using protein sequence similarity we identified two β-IDH encoding genes in *A. nidulans*: *leuD* and *leuE*. We have deleted these genes by gene replacement and shown that *leuD*Δ, but not *leuE*Δ, causes a leaky leucine auxotrophy. A *leuD*Δ *leuE*Δ double mutant is a strict leucine auxotroph and therefore both genes encode functional enzymes. Using quantitative RT-PCR we showed the difference in phenotypes is likely due to higher expression of *leuD* than *leuE*. We have also identified six putative BAT encoding genes, *batA-F*, by protein sequence similarity to Bat1p and Bat2p from yeast. Deletion of these six genes separately does not result in BCAA auxotrophy. However, simultaneous deletion of the two most highly expressed genes *batA* and *batB* is sufficient to confer BCAA auxotrophy, suggesting that *batC-F* may have evolved new roles within *A. nidulans* since duplication. With the identification of *leuD*, *leuE*, *batA* and *batB* the genes encoding the final steps of branched chain amino acid biosynthesis in *A. nidulans* have now been elucidated.

47. Detoxification of 5-hydroxymethylfurfural (HMF) by the *Pleurotus ostreatus* lignolytic enzymes aryl alcohol oxidase and dehydrogenase. D. Feldman[1], D. Kowbel[2], L. Glass[2], O. Yarden[1], Y. Hadar[1]. 1) Department of Plant Pathology and Microbiology, The R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 2) Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, California 94720, USA.

Current large scale pretreatment processes for lignocellulosic biomass are accompanied by the formation of degradation products, such as 5-hydroxymethylfurfural (HMF). HMF inhibits the cellulolytic enzymes and the ethanol-producing yeast. Overcoming these toxic effects is a key technical barrier in the biochemical conversion of biomass feedstock to biofuels. *Pleurotus ostreatus*, a white rot fungus, has a variety of secreted proteins involved in lignin biodegradation that include phenol oxidases and peroxidases, as well extracellular H_2O_2-producing enzymes such as aryl-alcohol oxidases (AAO) and intracellular aryl alcohol dehydrogenase (AAD).

P. ostreatus was capable of metabolizing and detoxifying 30 mM of HMF within 48 hours to 2,5-bis-hydroxymethylfuran and 2,5-Furandicarboxylic acid, which subsequently allowed for normal yeast growth. The addition of HMF significantly elevated expression levels of AAOs (*aao1-6*) and AAD (*aad1*). Expression levels increased 30-300 fold by 24 hrs, and as fast as 30 min after exposure to HMF. Subsequently, the abundance of AAOs and AAD was increased in the presence of HMF. HMF metabolism was also followed *in vitro* with fungal extract pre-exposed to the compound. AADs activity was coupled with NADPH, and AAOs metabolized HMF while generating H_2O_2.

We conclude that AAO and AAD-encoding gene family members are part of the transcriptional and translational reaction to HMF. Functional redundancy exists between the genes and their products, even though their roles in the reaction may differ. We propose that *P. ostreatus* can serve as gene pool for heterologous expression in yeast, or be integrated in biomass pretreatment.

48. Manganese ion deficiency plays a pivotal role in the itaconic acid production of *Aspergillus terreus*. Rafael Díaz[1], Benedek Papp[2], Erzsébet Fekete[1], Dávid Andrási[2], Erzsébet Sándor[2], Christian P. Kubicek[3], Levente Karaffa[1]. 1) Biochemical Engineering, University of Debrecen, Debrecen, Hungary; 2) Institute of Food Science, University of Debrecen, H-4032 Debrecen, Hungary; 3) Austrian Centre of Industrial Biotechnology, A-8010 Graz, Austria.

Itaconic acid (IA) is an unsaturated dicarboxylic acid. The conjunction of the two carboxyl groups and the methylene group that is able to participate in polymerization reactions makes it a potential new platform chemical derived from sugars.

IA is mostly produced by large-scale submerged fermentation by *Aspergillus terreus*. Although the biochemical pathway and the physiology leading to IA is almost the same as that leading to citric acid in *A. niger*, both the volumetric (g/L) and the specific yield (g/g carbon source) of IA are by far lower than for citric acid. Citric acid is known to accumulate to high levels only when a number of nutritional parameters are carefully adjusted, but these are not used in research on IA production. Two of those parameters are the concentration of the carbon source (D-glucose) and the concentration of Mn ions in the medium. We have here investigated the effect of variation in these parameters on IA production by *A. terreus*: we show that Mn (II) concentrations above 3 ppb decrease IA production in a concentration-dependent manner, with 1.000 ppb resulting in less than 40 percent of the volumetric yield achieved under identical conditions. We also provide evidence that increasing the D-glucose concentration increases the specific yield of IA. Both findings are in agreement with the effect of these parameters on citric acid production by *A. niger*, thus showing that the transfer of its fermentation technology to *A. terreus* and IA production can be used to arrive at high yields of this acid.

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49. RNA interference mediated suppression of ornithine decarboxylase to investigate polyamine metabolism in *Cryphonectria parasitica*. M. Pokharel, A.L Dawe. Molecular Biology Program and Department of Biology, New Mexico State University, Las Cruces, NM.

RNA interference (RNAi) machinery has been known as a valuable tool for deciphering the essential vital functions of genes in many eukaryotes, including fungi. This study involves targeting an important gene of polyamine biosynthesis pathway, ornithine decarboxylase (*odc*), by expression of hairpin RNA in *Cryphonectria parasitica. C. parasitica* is a filamentous fungus that can be infected by hypoviruses, which cause altered metabolism, development and virulence-attenuation of the fungus. Previous work has shown that the polyamine synthesis pathway was significantly altered, with spermidine accumulating to a 50-fold greater relative amount in the presence of hypovirus. To better understand the role of the polyamine pathway in *C. parasitica*, we examined the effects of the addition of 1,4-diaminobutanone (DAB), an *odc* inhibitor. We observed a reduction of mycelial growth and conidiation, which was reverted by addition of exogeneous putrescine. 1mM exogeneous putrescine was shown to be enough to revert 0.5mM DAB treated fungi in virus free strain, however the virus infected strain required as high as 6mM putrescine. Attempts to knock out the *odc* gene were not successful, possibly suggesting that its absence is lethal. To develop an RNAi approach, we used an expression vector that utilizes a copper regulated inducible promoter system to control the hairpin RNA expression. Similar to the DAB treatment, transformants expressing the odc-RNAi construct showed reduced mycelial growth and conidiation under inducing conditions and could be reverted by addition of exogenous putrescine. To further confirm the targeted effect of the RNAi expression, testing of the construct is also underway in strains that lack key components of the RNA silencing mechanism. This study demonstrates an essential role for ornithine decarboxylase in mycelial growth and conidiation in *C. parasitica*.

50. Transport proteins for itaconic acid production in *Aspergillus niger*. Matthias G. Steiger[1,2,4], Peter J. Punt[2,3], Arthur F. J. Ram[2], Diethard Mattanovich[1,4], Michael Sauer[1,4]. 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria; 2) University Leiden, Institute of Biology, Sylviusweg 72, 2333 BE Leiden, The Netherlands; 3) TNO Microbiology and Systems biology, Zeist, AJ 3700, The Netherlands; 4) University of Natural Resources and Life Sciences, Department of Biotechnology, Muthgasse 18, 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 be produced 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.

In order to analyze the influence of compartmentalization on 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 *A. terreus cadA* gene enables the formation of itaconic acid in *A. niger* although only low titers are obtained. An increase of the productivity was obtained by targeting the pathway to the mitochondria. Furthermore, it was shown that the heterologous expression of two transport proteins which are found in close proximity to the *cadA* gene in *A. terreus*, have a positive impact on the itaconic acid formation.

These two transport proteins are now characterized in more detail making use of an inducible promoter system. Their localization in the cell is determined tagging the proteins with GFP and fluorescence microscopy. Furthermore, the substrate specificity of the mitochondrial transporter is elucidated by a rearrangement of synthetic metabolic pathways.

51. Fungal cell factories for the production of biochemicals in biorefineries. L. Yang, M. Lübeck, P. Lübeck. Aalborg University, Copenhagen, Copenhagen SV, Denmark.

Currently there is a growing demand for sustainable production of biochemicals that substitute fossil based chemicals. Filamentous fungi are of great interests as biocatalysts in biorefineries as they naturally produce and secrete a variety of different organic acids that can be used as building blocks in the chemical industry. Aspergilli, as biotechnology workhorses, have great potentials as cell factories for production of organic acids. Strains of the black *Aspergillus carbonarius* naturally produce citric acid and gluconic acid in high amounts and have vast abilities in utilizing a broad range of substrates. The fungus has excellent tolerance to stress conditions and therefore is considered as a potential biocatalyst that could be used in lignocellulosic biorefineries. Ideally, by utilizing both its large potentials for secretion of hydrolytic enzymes and of organic acids, the fungus could be considered in a consolidated approach where it hydrolyses the plant biomasses and ferments the resulting sugars into different organic acids. However, for developing the fungus into an efficient biocatalyst for biochemical production, it is necessary to include metabolic engineering of biochemical pathways for increasing the glucose and xylose uptake and flux, and direct the carbon towards production of the selected organic acids. In our project, engineering of selected genes in the glycolytic pathway and in the pentose phosphate pathway have led to increased citric acid production. Furthermore, the effects of deleting the gluconic acid producing pathway and inserting an alternative cytosolic pathway on organic acid production were also evaluated. The impact of these genetic modifications on organic acid production will be presented.

52. Response to nitrogen limitation in *Yarrowia lipolytica*. Kyle Pomraning, Siwei Wei, Young-mo Kim, Carrie Nicora, Therese Clauss, Rosalie Chu, Sam Purvine, Ziyu Dai, Erin Bredeweg, Dehong Hu, Sue Karagiosis, Richard Smith, Galya Orr, Thomas Metz, Scott Baker. Pacific Northwest National Laboratory, Richland, WA.

Yarrowia lipolytica is an oleaginous ascomycete yeast that accumulates high levels of triglycerides in a specialized organelle called the lipid body. Fatty acid methyl esters derived from triglycerides and other lipids are liquid fuel precursors making *Y. lipolytica* a useful organism for the production of renewable bioenergy. Lipogenesis can be induced by limitation of nitrogen but the metabolic pathways

leading to this response have not been thoroughly characterized in *Y. lipolytica*. We have analyzed Yarrowia's response to nitrogen limitation in batch cultures using proteomics, phospho-proteomics and metabolomics to deepen our understanding of how this organism regulates and controls lipid metabolism. Initial results have identified shifts in metabolite pools between wild-type *Y. lipolytica* growing in high (C/N = 10) or low (C/N = 150) nitrogen conditions as well as a number of phosphorylated proteins involved in lipid metabolism, including Acetyl-CoA Carboxylase which catalyzes an important step in *de novo* biosynthesis of long-chain fatty acids. We have constructed strains and vectors for replacing, overexpressing, and GFP tagging proteins in *Y. lipolytica* and are putting them to use analyzing the function of genes implicated in nitrogen limitation induced lipogenesis by these -omics experiments.

53. Evidences that a secondary metabolite is the Fe^{3+}-reductant secreted by an ectomycorrhizal fungus during decomposition of litter material. Firoz Shah[1], Daniel Schwenk[2], César Nicolás[1], Per Persson[1], Dirk Hoffmeister[2], Anders Tunlid[1]. 1) MEMEG, Department of Biology, Lund University, Lund, Sweden; 2) Department of Pharmaceutical Biology at the Hans-Knöll Institute, Friedrich-Schiller-Universität, Jena, Germany.

Ectomycorrhizal (ECM) fungi are thought to play a key role in mobilizing nutrients embedded in recalcitrant organic matter complexes and thereby making them accessible to the host plant. Recent work combining spectroscopic analyses with transcriptome profiling have indicated the oxidative degradation of litter extract involving Fenton chemistry ($Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O$) by the ECM fungus *Paxillus involutus*. In many wood-degrading brown-rot fungi, secreted metabolites are one of the component which drive one-electron reductions of Fe^{3+} and O_2, generating Fenton reagents. We therefore investigated if such compounds are also produced by the ECM fungus *P. involutus* during litter degradation. Activity-guided purification was performed to isolate the Fe^{3+}-reducing compound (s) secreted by *P. involutus* during growth on organic matter extract. The Fe^{3+}- reducing activity correlated with the presence of one compound. Mass spectrometry and NMR identified this compound as the diphenylcyclopentenone pigment involutin. A major part of the involutin produced by the fungus during organic matter decomposition was secreted into the medium and the pigment was not detected when the fungus was grown in a mineral nutrient medium. We also found that under physiological concentration of H_2O_2, involutin has the capacity to drive an *in vitro* Fenton reaction. Our results suggest that mechanisms for generating the Fenton reagents by secreted metabolites are similar in ECM fungi and saprophytic brown-rot fungi. It remains to be determine whether involutin can reduce Fe^{3+} from mineral complexes in natural soil systems or if cooperative processes including other metabolites are required.

54. The ORF NCU08772 encodes a putative multifunctional cyclin involved in germination, cell cycle regulation, germination and glycogen metabolism in *Neurospora crassa*. Thiago de Souza Candido, Fernanda Barbosa Cupertino, Maria Célia Bertolini. UNESP - IQ - Araraquara, SP.

Neurospora crassa has been widely used as a model organism for fundamental aspects of eukaryotic biology. In a Blast search, we found the *Neurospora crassa* NCU08772 ORF product as the *Saccharomyces cerevisiae* Pcl10p cyclin homologue having (32% of identity) at protein level. In *yeast*, the Pcl10p protein, together with the Pho85p cyclin-dependent protein kinase, phosphorylates glycogen synthase, the regulatory enzyme in glycogen synthesis. Phosphorylation results in the enzyme inactivation and, therefore, decreaseding the glycogen accumulation storage. The *pcl-1*^{KO} strain showed a delay in germination and a shift in the timing of cell division when compared to the wild-type strain suggesting that this cyclin regulates fungal development and cell division. The knocked out strain showed accumulated higher levels ofin the glycogen than the wild-type strain accumulated during growth than the wild-type strain. The glycogen synthase (GSN-1) phosphorylation, and its activation state, was monitored by the –G6P/+G6P activity ratio, higher levels correlating with greater activity. The mutant strain showed lower phosphorylation, and then higher activity during growth, a result that explains the hyper-accumulation of glycogen. The GSN-1 phosphorylation profile in the mutant strain was analyzed by 2D-PAGE followed by Western blot. GSN-1 presented less phosphorylated isoforms than the wild-type strain. The *N. crassa* recombinant proteins PHO85, PCL-1 and GSN-1 proteins were produced in *E. coli* and used in *in vitro* phosphorylation assays. GSN-1 was phosphorylated by PHO85/PCL-1 and determined the putative phosphorylation site (Ser636). The expression of the genes encoding the enzymes involved in synthesis and degradation of glycogen was analyzed by qPCR. There were no significant differences in gene expression between the two strains indicating that the difference in glycogen levels was a consequence of the phosphorylation state of GSN-1 in the mutant strain and not in the gene expression. Supported by FAPESP, CNPq.

55. Metabolism of the chitin monomer *N*-acetylglucosamine in *Neurospora crassa*. R. Gaderer, L. Kappel, V. Seidl-Seiboth. Vienna University of Technology, Vienna , Austria.

Chitin is not only an important structural component of the fungal cell wall but also the second most abundant biopolymer on earth. Filamentous fungi have a multitude of chitinases for chitin degradation. The monomer GlcNAc is intracellularly converted into fructose-6-phosphate, but the catabolism of GlcNAc has so far only been investigated in bacteria and the yeast *Candida albicans*, but not in filamentous fungi. We found that in filamentous fungi the genes involved in GlcNAc catabolism are clustered and that this cluster contains an NDT80-like transcription factor, which is not present in *C. albicans*. *N. crassa* has three NDT80-like transcription factors. Two of them, VIB1 and FSD1 are involved in sexual development and programmed cell death. The gene for the third one, which we named *ron-1* (regulator of N-acetylglucosamine catabolism), is located in the GlcNAc catabolism gene cluster and so far no phenotype or overlap in function with either *fsd-1* or *vib-1* was detected. Moreover a GH 3-family gene with proposed β-*N*-acetylhexosaminidase activity is present in the cluster. Growth assays showed that, in contrast to other fungi, GlcNAc is surprisingly a very poor carbon source for *N. crassa*. GlcNAc turned even out to be growth-inhibiting upon in the presence of other carbon sources. Nonetheless *N. crassa* was still able to grow on chitin, probably due the slow release of GlcNAc from this polymer, leading to non-inhibitory concentrations. We performed an analysis of knockout mutants of the GlcNAc catabolism cluster genes. GlcNAc-6-phosphate deaminase has been reported to be a rate-limiting step in bacteria. The lack of this gene also completely abolished growth of *N. crassa* on GlcNAc-containing media, which can probably be attributed to deleterious effects of GlcNAc-6-phosphate. Complementation of this mutant with the GlcNAc-6-phosphate deaminase from *Trichoderma reesei* restored growth on GlcNAc to *N. crassa* wild-type levels, but not beyond that, which suggests that other steps are rate-

limiting for the inability of *N. crassa* to metabolize GlcNAc efficiently.

56. The RACK1 homolog CPC-2 regulates cross pathway control via post-translational regulation of the bZIP transcription factor CPC-1 in *Neurospora crassa* . Arit Ghosh, Amruta Garud, Katherine Borkovich. Plant Pathology & Microbiology, University of California, Riverside, Riverside, CA.

Cross pathway control or general amino acid control is a global response mechanism by which eukaryotic cells can respond to changes in amino acid starvation. Depletion of one amino acid can lead to upregulation of various enzymes vital to other amino acid biosynthetic pathways. In the filamentous fungus *Neurospora crassa*, derepression of the bZIP transcription factor *cpc-1* (*cross pathway control-1*) is essential for transcriptional induction of amino acid biosynthetic genes when cells are artificially starved for histidine through supplementation with 3-aminotriazole. The WD40 protein/ RACK1 homolog- encoding gene – *cpc-2* (*cross pathway control-2*) has also been found to be an important component of the cross pathway network in *N. crassa*, with added functions in female fertility and negative control of asexual sporulation. We have found that CPC-2 plays a positive role in activating general amino acid control under starvation conditions in *N. crassa*, as deletion of *cpc-2* blocks transcriptional induction of amino acid biosynthetic genes – *arg-3, trp-3* and *his-3* - under starvation. Interestingly, these genes are de- repressed under non-starvation conditions in the *cpc-2* deletion mutant. Additionally we have detected a putative higher molecular weight form of CPC-1 protein using western analyses. Although *cpc-1* transcript levels are elevated in the *cpc-2* knockout mutant, the higher molecular weight form of CPC-1 protein is down regulated. Also, *cpc-1* mRNA is present on higher molecular weight polysomes in both wild type and the *cpc-2* knockout mutant. This suggests that CPC-2 mediated regulation of CPC-1 might occur at the post- translational level.

57. Understanding pathway based auxin biosynthesis and transport in filamentous fungi through structure-function correlation and its evolutionary consequences. Puspendu Sardar, Frank Kempken. Genetics & Molecular Biology in Botany, Institute of Botany, CAU-Kiel, Kiel, Germany.

The phytohormone auxin (IAA) can be synthesized from the amino acid tryptophan (Trp) via different pathways. The YUCCA (YUC), the indole-3-pyruvic acid (IPA), the indole-3-acetamide (IAM), and the indole-3-acetaldoxime (IAOx) are four proposed pathways for biosynthesis of IAA from Trp in green plants [1]. While auxin production in filamentous fungi has been described earlier [2], the exact pathway for IAA production is poorly understood so far and so is auxin transport. Tryptophan aminotransferase (TAM), pyruvate decarboxylase (IPD), indole-3-acetaldehyde dehydrogenase (IAD) and YUC are important intermediate proteins involved in auxin biosynthesis pathways in green plants. Employing a computational approach we examine the involvement and putative role of above mentioned enzymes in auxin biosynthesis pathway and transport in filamentous fungi. In addition we analyze structure-function conservation throughout the evolution. For this study we have used the filamentous fungus *Neurospora crassa* as the focal species. Primary results suggest that *tam1, ipd, iad1* and *yuc* genes are present in a wide range of fungi across the fungal kingdom. Moreover gene NCU00589.7 present in *N. crassa* has a homolog in *Arabidopsis thaliana* with a similar annotation as "auxin efflux carrier family protein". In particular, we have studied the structure-function conservation and correlation of the encoded proteins of the candidate genes using homology modeling in addition with specificity in ligand binding site.

References:
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58. Reserve carbohydrate metabolism is controlled by pH signaling pathway in *Neurospora crassa*. Stela Virgilio, Fernanda Barbosa Cupertino, Maria Célia Bertolini. Instituto de Química, UNESP, Araraquara, São Paulo, Brazil.

Extracellular pH has an important role in cell biology as it regulates gene expression and consequently influences a variety of processes. The regulation of gene expression by pH has been studied in *Aspergillus nidulans* in which the regulator PacC is firstly activated by the pH-dependent *pal* genes cascade followed by a second proteasome-mediated proteolytic cleavage and pH-independent. The *N. crassa* genome has the six *A. nidulans pal* gene homologues involved in pH regulation. Recently, the motif sequence of the *N. crassa* PAC-3 (5'-BGCCVAGV-3'), the PacC homologue, was identified by PBM (Protein Binding Microarrays, *Cell* 2014) and the motif was found in the promoters of genes involved in the glycogen and trehalose metabolism. Here we describe the characterization of the *N. crassa pal* genes and their participation in the glycogen and trehalose metabolism regulation. The *pal* knocked out strains showed high melanin production and normal growth at pH 5.8. However, they were unable to grow at alkaline pH (7.8) confirming that the *N. crassa pal* genes are involved in the pH-signaling pathway. The expression of some *pal* genes was influenced by pH 7.8 and regulated by PAC-3. ChIP-qPCR analysis demonstrated that PAC-3 bound to *pal* promoters under alkaline pH confirming their regulation by PAC-3. The levels of the reserve carbohydrates glycogen and trehalose were quantified in all mutant strains and compared to the wild-type strain. Both carbohydrates were accumulated at higher levels in all mutant strains, except in *pal-9*, in pH 5.8. However, the glycogen accumulated was even higher up to 4 h of transferring to alkaline pH while the trehalose levels were the same in the mutant and wild-type strains after 1 h of transferring. The expression of glycogenic genes was either up- or down-regulated by alkaline pH in the wild-type strain. However, PAC-3 only bound *in vivo* to the glycogenic gene promoters in pH 7.8. These results indicate that the *N. crassa pal* genes play a role in the pH-signaling pathway, leading to the PAC-3 activation and regulation of glycogen metabolism by PAC-3. Experiments are currently underway to investigate whether PAC-3 regulates trehalose genes in normal and alkaline pH. Supported by FAPESP and CNPq.

59. Comparative analysis of the sterol biosynthetic pathways in oomycetes. P. Dahlin, V. Bulone, S. Ekengren. Royal Institute of Technology , Stockholm, Sweden.

The oomycete phylum comprises plant and animal pathogens. Sterol requirements vary significantly between oomycete orders, which reflects different biosynthetic pathways. These variations can potentially be exploited to devise group-specific processes for disease

control. We have initiated a comparative analysis of the sterol metabolic pathways in two economically important species from the Peronosporales and Saprolegniales orders, *i.e. Phytophtora infestans* and *Saprolegnia parasitica*, respectively. Bioinformatic and compositional analyses were combined to reconstruct the sterol biosynthetic pathways of both organisms. Ten enzymes involved in the biosynthesis of desmosterol, lanosterol, 24-methylenecholesterol and cholesterol were identified in *S. parasitica* and the expression of the corresponding genes was analyzed by quantitative PCR. The occurrence of these sterols in *S. parasitica* was demonstrated by metabolite analysis and supported by the PCR data. The genes exhibiting the strongest expression in the mycelium encode putative sterol-C24-methyltransferases, whereas C-3 sterol dehydrogenases showed the lowest expression levels. The gene encoding CYP51, a known target of azoles in fungi, was expressed at an intermediate but relatively high level. Orthologous genes with the same predicted function were identified in other Saprolegniales species, thereby pointing to a common biosynthetic pathway in this order. Interestingly, putative genes coding for a C5 sterol desaturase and a C7 reductase were identified and highly expressed in the mycelium of *P. infestans*, although this species is not able to synthesize sterols *de novo*. The function of the latter enzymes is being determined through metabolite analysis of *P. infestans* grown in the presence of intermediates of sterol biosynthetic pathways. Preliminary results show that lathosterol and lanosterol are converted into cholesterol and zymosterol *in vivo*, hence supporting the occurrence of active sterol-modifying enzymes in *P. infestans*. Out of the 12 sterols tested in culture media, cholesterol only was converted into sterol-glycosides, thereby pointing to a possible important physiological role of glycosylated sterols in Peronosporales.

60. How menadione triggers aflatoxin synthesis in *A. flavus*. M. Zaccaria[1], M. Ludovici[1], M. Scarpari[1], V. Scala[1], A.A. Fabbri[1], C. Fanelli[1], W. Sanseverino[2], M. Reverberi[1]. 1) Environmental Biology, University of Rome , Roma, Roma, Italy; 2) Sequentia Biotech, carrer comte d'Urgell, Barcelona, Spain.

Aspergillus flavus is a saprophytic fungus responsible for world-wide spread harvest and post-harvest infections on cultivations, mainly on cereal grains and legumes. *A. flavus* is one of the main producers of aflatoxin B1, the most dangerous carcinogenic metabolite in nature. In relation to this, ongoing climate changes favor plant susceptibility to the attack by this fungus with a consequent, dangerous increase of aflatoxins into previously unexploited feed and foodstuff. In order to address effectively the economic and sanitary consequences of *A. flavus* contamination, a detailed and extensive knowledge of the pathogen metabolism and of the environmental conditions triggering the different biological processes, is of paramount importance.

In this study, we focus on the effects of oxidative stress in the intracellular compartment, obtained by adding menadione 0,1mM to the culture medium. Menadione is a chinone and a precursor of vitamin K, its cytotoxicity has been extensively investigated in several human and murine cellular lines as an intracellular ROS inductor. Chinones are very common in nature. As substrates for flavoenzymes they may incur in one electron reduction to semichinone which, conversely, reduce O_2 to superoxide anion in the intracellular environment, therefore providing a stressing condition. We evaluate the response from *A. flavus via* several analytical approaches: mycelial growth, conidia quantification, aflatoxin B1 synthesis, antioxidant enzymes activity, intracellular ROS quantification. To evaluate gene expression, we exploit RNAseq technology for transcriptome analysis, plus RT-PCR of markers for cellular respiration, pentose phosphate pathway, and oxidative stress response and sirtuins expression. Lastly, we have extracted and evaluated oxylipins by an MRM based LC-MS/MS method, in order to ascertain if they may represent a more stable reactive signal able to trigger aflatoxin synthesis and conidiogenesis through a remodulation of *A. flavus* metabolism in oxidative stress conditions.

61. Kinase activity of HwHog1 is essential for survival of *Hortaea werneckii* in solar salterns. A. Kejžar[1], M. Grötli[2], M. J. Tamás[2], M. Mihelič[3,4], D. Turk[3,4], A. Plemenitaš[1], M. Lenassi[1,3]. 1) Institute of Biochemistry, Faculty of Medicine, Ljubljana, Ljubljana, Slovenia; 2) Department of Chemistry and Molecular Biology, University of Gothenburg, S-405 30 Gothenburg, Sweden; 3) Department of Biochemistry and Molecular and Structural Biology, Jozef Stefan Institute, SI-1000 Ljubljana, Slovenia; 4) Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Jamova 39, SI-1000 Ljubljana, Slovenia.

Extremelly halotolerant fungus *Hortaea werneckii* employs the compatible solute strategy to counteract the devastating effects of hyperosmolar environment typical for the solar salterns. In this study, we addressed the role of HOG signalling pathway in *H. werneckii* survival to osmotic stress by inhibiting the HwHog1 kinase activity with the ATP analogue BPTIP. Based on genetic information, HOG pathway in *H. werneckii* is very complex and robust. As shown by functional complementation and expression studies, it includes two functionally redundant MAPK homologues, HwHog1A and HwHog1B, which show osmolyte-type-dependent phosphorylation. Their activities are effectively inhibited by BPTIP, which was shown by following growth of *S. cerevisiae Δhog1* strain expressing HwHog1A/B, and by *in vitro* fluorescent coupled kinase assay on purified proteins. Inhibition of HwHog1 kinase activity with BPTIP restricted *H. werneckii* colony growth at 3.0 M NaCl, KCl and sorbitol, most likely due to restricted cell division. On the other hand, we have demonstrated that HwHog1-regulated transcription of a selected group of genes is an osmolyte-specific process. While inhibition of HwHog1 kinase activity affected transcription of a selected group of Hog1-dependent genes at high salt concentration (4.5M NaCl), no effects on transcription were observed when KCl was used. Our results also showed that HwHog1 activity is necessary for induction of transcription of target genes at 3.0 M concentration sorbitol. Altogether we have demonstrated, that HOG pathway, which senses the osmolyte type and is capable of regulating common and osmolyte-specific processes, is vital for the extreme osmotolerance of *H. werneckii* and its survival in environments with extremely high NaCl concentrations.

62. Propionate metabolism in *Paracoccidioides* sp.: proteomic and biochemistry analysis. Luiz Paulo Araujo Santos[1], Patrícia de Souza Lima[1], Matthias Brock[2], Leandro do Prado Assuncao[1], Clayton Luiz Borges[1], Celia Maria de Almeida Soares[1], Alexandre Melo Bailao[1]. 1) Molecular Biology Dep, Federal University of Goiás, Brazil; 2) Laboratorium für Mikrobiologie, Philipps-Universität, Marburg, Germany.

Some microorganisms utilize propionate as the carbon source. Pathogenic fungi may found this metabolite or propionate generating sources during infectious process. In some microorganisms, the methylcitrate cycle (MCC) is responsible for propionyl-CoA conversion into pyruvate. However, there are no studies on this pathway in the fungi *Paracoccidioides* spp. It has been shown that specific enzymes of

MCC are upregulated in infection-mimicking conditions. *In silico* analyses revealed that the fungus presented the three enzymes related to MCC and two of them are next to each other in the genome. It was observed that the fungus is able to metabolize and growth in propionate containing medium. The genes encoding for methylcitrate synthase, methylcitrate lyase, methylcitrate dehydratase and propionyl-CoA ligase were up-regulated in propionate containing medium when compared to glucose. The MCS activity in propionate was higher than in glucose. The mycelium phase present higher MCS activity then yeast phase and MCS activity was detected in culture supernatant. The recombinant enzyme displayed both, citrate and methylcitrate synthase activity, suggesting its role MCC. Moreover, the Km determination of the MCS confirmed its higher affinity to propionyl-CoA than to acetyl-CoA. The proteomic analysis during incubation in propionate, glucose plus propionate and glucose only was assayed. The proteomic data shown an increasing in enzymes related to propionyl-CoA metabolization and in molecules related to alternative carbon source utilization. Several enzymes related to gluconeogenesis, fatty acid oxidation and aerobic metabolism. Corroborating, alcoholic fermentation is downregulated in propionate. Given that methylcitrate cycle is responsible for propionyl-CoA detoxification in the fungal cells and the study of this pathway in *Paracoccidioides* spp. will provide data for comprehension of metabolic adaptation that this fungus takes hand in different host niches and also in nature.

63. Comparisons of the responses of fungi to lignocellulosic substrates using single and mixed cultures. Paul Daly[1], Matt Kokolski[1], Juliana Velasco de Castro Oliveira[2], Gustavo Borin[2], Martin Blythe[1], Gustavo Goldman[2,3], David Archer[1]. 1) University of Nottingham, Nottingham, United Kingdom; 2) CTBE, Campinas, São Paulo, Brazil; 3) Universidade de São Paulo, São Paulo, Brazil.

Fungi are the main source of enzymes used for saccharification of lignocellulose. The efficiency of saccharification can be improved by better understanding the responses of fungi to lignocelluloses. We investigated the responses of *Trichoderma reesei*, *Aspergillus niger* and *Penicillium chrysogenum* to lignocelluloses partly with the aim of establishing conditions for a transcriptomic study of mixed cultures using Dual-RNAseq.

For single culture experiments, supernatants were sampled for proteomics from a time-course of shake-flask cultures with bagasse or wheat straw. For mixed culture experiments, mycelia from single species glucose-grown pre-cultures were combined for straw cultures and qPCR of the ITS region from each fungus was used to quantify species-specific fungal RNA.

In the comparison of the proteomics responses of the fungal single cultures to straw or bagasse, there were more proteins significantly different in abundance between the *T. reesei* straw and bagasse cultures than between the *A. niger* straw and bagasse cultures. This could partly be due to a later secretory response of *T. reesei* to straw compared to bagasse whilst *A. niger* secreted proteins at similar times on both substrates. In the mixed cultures, over a period of 24 h, the relative amounts of RNA from each fungus in two species mixed cultures of either *A. niger* and *P. chrysogenum* or *T. reesei* and *P. chrysogenum* didn't change substantially. In contrast, the relative amount of *A. niger* RNA declined in the mixed culture with *T. reesei.*

Differences in gene content or regulation between *A. niger* and *T. reesei* could explain the delay in secretion of enzymes by *T. reesei* on straw compared to bagasse single cultures. Mixed cultures of *P. chrysogenum* with either of the other two fungi are likely to be more suitable to study gene expression in lignocellulose mixed cultures with limited antagonism than mixed cultures of *A. niger* and *T. reesei*. (Financial support: FAPESP, Brazil and BBSRC, UK.).

64. Secreted protein profile and gene expression analysis of *Trichoderma harzianum* induced with different phytopathogen cell walls. Marcelo Ramada[1,2], Andrei Steindorff[1,3], Carlos Bloch Jr.[2], Cirano Ulhoa[4]. 1) Brasilia University, Brasilia, DF, Brazil; 2) Mass Spectrometry Lab, Embrapa Cenargen, Brasilia, DF, Brazil; 3) DOE Joint Genome Institute, Walnut Creek, CA, USA; 4) Enzymology Lab, Federal University of Goias, Goiania, GO, 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 oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, phytopathogen fungi that causes several losses around the world in different crops, in a dual culture assay and to evaluate the secreted proteins of *T. harzianum* ALL42 when its spores were inoculated and incubated for 48 hours in culture media (TLE) supplemented with *F. oxysporum*, *R. solani* or *S. sclerotiorum* cell walls (FoCW, RsCW, SsCw, respectively). *T. harzianum* was able to control the phytopathogens growth and started to sporulate in its area after 7-10 days, indicating that it had successfully parasitized the host. Protein quantification showed that TLE+FoCW, TLE+RsCW and TLE+SsCW had 49,86, 84,25 and 84,12 µg mL^{-1}, respectively. The difference between protein profiles was observed in the bidimensional gels, as the media supplemented with phytopathogen cell walls showed a complex profile (around 200 spots each) but with many similar proteins, based on their molecular weight and isoeletric point. The bidimensional gel of a previous study using *Fusarium solani* cell walls (FscW) or glucose (GLU) as the inducing sources were used as a control. A total of 93 proteins were excised from all three conditions and submitted to mass spectrometry analysis, manual *de novo* interpretation of MSMS spectra and further search in proteins database. 76 out of 93 proteins were successfully identified, as 27 were not identified in our previous study. This 27 proteins were the product of 16 different genes. Gene expression analysis was performed by RT-qPCR and confirmed some of the results obtained by bidimensional gels. *T. harzianum* ALL42 mainly secretes a common set of proteins when induced by different cell walls as well as proteins that seems to be exclusive for each condition, as confirmed by RT-qPCR.

65. Comparative proteomic and phenotypic analysis of *Paracoccidioides lutzii* isolated in a fatal case of fungemia. P. Martins[1], L. Casaletti[1], C. Borges[1], M. Silva-Bailao[1,2], R. Hahn[3], C. Soares[1], A. Bailao[1]. 1) Federal University of Goiás, Institute of Biological Sciences, Goiânia, Goiás, Brazil; 2) Federal University of Goiás, Jataí Campus, Medical College, Jataí, Goiás, Brazil; 3) Federal University of Mato Grosso, College of Medical Sciences, Cuiabá, Mato Grosso, Brazil.

Fungemia corresponds to the presence of fungi in the bloodstream and is most commonly observed in patients with an impaired immune

system. The presence of fungi in the blood can cause life threatening infections, becoming a serious health problem. Few studies have detected *Paracoccidioides* spp. in circulating blood of patients. These fungi are the etiological agents of paracoccidioidomycosis, a systemic mycosis characterized by a chronic granulomatous inflammation, whose incidence is high in Latin America. In the present study, *Paracoccidioides lutzii* isolated from a fatal case of fungemia in an immucompetent patient was examined. In order to identify the molecular factors related to this specific phenotype, a comparative proteomic analysis was performed. The samples were analyzed by nanoscale liquid chromatography coupled with tandem mass spectrometry (nanoUPLC-MS^E), where soluble proteins from fungemia strain (PbF) were compared with proteins of another *P. lutzii* isolate, Pb01. Survival of PbF in blood and its growth on blood BHI plates were also achieved. The phenotypic characterization tests in blood revealed a better adaption of fungemia isolate in this environment, when compared to Pb01. NanoUPLC-MS^E approach allowed the identification of 470 proteins of PbF, of which 151 were up-regulated and 136 were down-regulated. The most representative categories, according to FunCat2, in both groups were related to metabolism, followed by protein synthesis and energy. Enzymes related to glucose utilization by anaerobic pathway, such as alcohol dehydrogenase and pyruvate decarboxylase, were induced in PbF. Enzymes related to the oxidative stress, such as thioredoxin reductase, were also induced. This work proposed the molecular characterization of a fungemia strain and may help in the elucidation of the virulence mechanisms used by the fungus during hematogenous dissemination.

66. Contributions of the nitrate assimilation pathway to *Phytophthora infestans* revealed by transcriptional profiling and gene silencing. Melania Abrahamian, Howard Judelson. Department of Plant Pathology & Microbiology, University of California, Riverside, CA.

Phytophthora infestans is a devastating potato and tomato pathogen of historic and contemporary significance. Little is known of how the metabolism of *P. infestans* adapts to different growth conditions and host tissues. To accomplish this, we have annotated metabolic genes within the *P. infestans* genome and used RNA-seq to follow their expression in cultured media or in infected potato tubers and tomato leaves. Many pathways were found to be expressed differentially on different host tissues, including those for the uptake and assimilation of nitrate. To help understand the importance of these genes in *P. infestans*, we generated stable knockdown strains using homology-based silencing, and studied the effect of silencing during growth on artificial media and *in planta*. The results indicate that the nitrate assimilation pathway makes an important contribution to the fitness of *P. infestans*, but only under certain conditions of growth or on host tissues.

67. Investigating terpenoid production in basidiomycete fungi. Alice M Banks, Andy M Bailey, Gary D Foster. Molecular Plant Pathology and Fungal Biology Group, School of Biological Sciences, University of Bristol, Bristol Life Sciences Building, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK.

Basidiomycetes are prolific producers of novel terpenoid compounds offering a diverse range of bioactivities including antibiotic, anti-inflammatory, anticancer, and anthelminthic among others. As a phylum, basidiomycetes have been largely neglected in natural product research but are likely to provide an extensive source of novel bioactive compounds.

There are two basidiomycete species which are the focus of this project: *Coprinopsis strossmayeri* and *Lepista sordida*. These were selected based on the high antimicrobial activity exhibited where *C. strossmayeri* inhibits the growth of *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*, and *L. sordida* inhibits the growth of *B. subtilis* and *E. coli*. There is also evidence in the literature that these species produce antimicrobials but little further investigation has been carried out.

Both genomes have been sequenced and analysed for genes characteristic of terpenoid production, in the search for gene clusters coding for novel compound biosynthesis. Seven core terpenoid synthases were identified in *C. strossmayeri* and 16 were identified in *L. sordida*. The genomic contexts of these genes were analysed to determine putative gene clusters, and qPCR analysis indicated genes which were highly expressed in conditions where antimicrobial agents were produced. Genes of interest have been taken forward to be used in heterologous expression to determine gene function. Phylogenetic trees have also been produced using genes in known fungal terpenoid pathways to make predictions on the core structure of these compounds.

Chemical analysis is ongoing to determine the products of biosynthetic pathways and other antimicrobial metabolites produced. Secondary metabolites and intermediate products from heterologous expression will be identified and screened for antimicrobial activity.

68. Diarylcyclopentenone pigment biosynthesis in *Paxillus involutus*. Jana Braesel[1], Gerald Lackner[1], Sebastian Götze[2], Firoz Shah[3], Anders Tunlid[3], Pierre Stallforth[2], Dirk Hoffmeister[1]. 1) Friedrich-Schiller-Universität Jena, Department Pharmaceutical Microbiology at the Hans-Knöll-Institute, Jena, Germany; 2) Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Junior Group Chemistry of Microbial Communication, Jena, Germany; 3) Lund University, Department of Biology, Lund, Sweden.

The basidiomycete *Paxillus involutus* is one of the best studied ectomycorrhizal fungi at the molecular, physiological, and ecological level. It is known to produce 2,5-diarylcyclopentenone pigments, among them involutin that likely serves as scavenger of free radicals. The biosynthesis of diarylcyclopentenones is unclear, as three routes appear possible that either include or circumvent atromentin, i.e., a central intermediate for numerous basidiomycete pigments.

We identified six genes (*invA1 – invA6*) in the genome of *Paxillus involutus* encoding tri-domain peptide synthetase-like enzymes. Biochemical analysis of the heterologously produced InvA enzymes proved atromentin synthetase activity for InvA1, InvA2 und InvA5. Combined evidence from biochemical *in vitro* enzyme characterization, transcriptomics, and feeding experiments to track the turnover of stable-isotope labeled precursors by *Paxillus involutus* suggested that the 2,5-diarylcyclopentenones are synthesized via atromentin as

metabolic intermediate. We also characterized the *Paxillus involutus* phosphopantetheinyl transferase PptA, which converts the above quinone synthetases from their inactive *apo*- into the functional *holo*-forms.

69. A new view on Aspergillus chemotaxonomy. Jens Frisvad. CMB, Dept Systems Biol, Kgs. Lyngby, Denmark.

After the nomenclatural changes (One Fungus One Name) accepted by the Botanical Congress in 2011, Aspergillus is preferred by most scientists for Aspergillus itself and all the former names used for the sexual state of these fungi and genera with asexual state that are included in Aspergillus sensu stricto according to DNA sequence data: Chaetosartorya, Cristaspora, Dichotomomyces, Emericella, Eurotium, Fennellia, Hemisartorya (?), Neocarpenteles, Neopetromyces, Neosartorya, Petromyces, Phialosimplex, Polypaecilum, Saitoa, and Sclerocleista. The Aspergillus genus and sections can be regarded as polythetic classes regarding morphology and exometabolites, i.e. most, but not all, species express a given feature, and each species would have those features in different combinations. Even though the sections are monophyletic, as judged by DNA sequences, the combination of features include symplesiomorphies, synapomorphies and on top of that autapomorphies, making a hennigian cladistic analysis of such phenotypic features irrelevant. In the closely related sections Flavi, Nigri and Circumdati, only one biosynthetic family of exometabolites, ochratoxins, is common. A detailed analysis of all the exometabolite biosynthetic families in the Aspergilli shows, however, that species in these sections have very similar kinds of exometabolites in common. For example di-diketopiperazines are produced by species in the three sections: Ditryptophenalins in Flavi, asperazines in Nigri and aspergamides in Circumdati. Such di-diketopiperazines are also produced in more distantly related species, for example eurocristatine in section Aspergillus (formerly Eurotium). Another example is the epidithiodioxopiperazines, which are represented by gliotoxin in Section Fumigati, by acetylaranotin in section Terrei, by emethallicins, emestrins and dithiosilvatins in Nidulantes and by aspirochlorin in Section Flavi. Sterigmatocystin is produced by species in the distantly related sections Flavi, Ochraceorosei, Nidulantes and Cremei. Thus Aspergillus is a genus in which the species have many common morphological and chemical features, despite some important differences in ecophysiology and sexual features.

70. A new type of melanin required for pigmentation of *Aspergillus terreus* conidia. E. Geib[1], Markus Gressler[1], Sandor Nietzsche[3], Christian Hertweck[2,4], Matthias Brock[1,4]. 1) Microbial Biochemistry and Physiology, Hans-Knoell-Institute, Jena, Germany; 2) Biomolecular Chemistry, Hans-Knoell-Institute, Jena, Germany; 3) Electron Microscopic Centre, University Hospital of the Friedrich-Schiller-University, Jena, Germany; 4) Friedrich-Schiller-University, Jena, Germany.

Aspergillus species produce pigmented asexual conidia. In the human pathogen *Aspergillus fumigatus*, this pigment has been shown to act as virulence determinant by inhibiting the acidification of macrophage phagolysosomes. Interestingly, the polyketide synthase (PKS) responsible for this pigment in *A. fumigatus* is also conserved in most other *Aspergillus* species leading to similar final products. An exception is *Aspergillus terreus*. BLAST analyses have shown that the respective PKS is lacking. However, pigmented conidia are produced. Therefore, the aim of our study was the analysis of secondary metabolite gene clusters that are expressed under sporulating conditions. Among the PKS several genes were expressed during vegetative growth on different culture media and during sporulation, whereas only the PKS essential for terretonin biosynthesis was exclusively active under sporulating conditions. This indicates that, in contrast to other Aspergilli, no PKS is involved in conidia pigmentation. A search for non-ribosomal peptide synthetases (NRPS) revealed several candidates that are expressed during sporulation. Beside the gene required for asterriquinone synthesis, other NRPS-like enzymes were identified for which a functional annotation is lacking. Targeted gene deletion resulted in white conidia with one of the NRPS-like enzymes, indicating that the melanin in *A. terreus* derives from the condensation of 2-oxoacids. Furthermore, deletion of a neighbouring gene led to fluorescent yellow conidia and structure elucidation of the compound is currently under way. In contrast to white conidia from *A. fumigatus*, scanning electron microscopy showed that the hydrophobin attachment on white *A. terreus* conidia is not affected. This indicates different properties of the *A. terreus* pigment compared to conidia pigments from other *Aspergillus* species.

71. Systems effects of gliotoxin *bis*-thiomethylation in *Aspergillus fumigatus*. Rebecca A. Owens, Stephen K. Dolan, Grainne O'Keeffe, Elizabeth B. Smith, Stephen Hammel, Kevin J. Sheridan, David A. Fitzpatrick, Sean Doyle, Gary W. Jones. Department of Biology, Maynooth University, Maynooth, County Kildare, Ireland.

Gliotoxin (GT) is a nonribosomally synthesized redox active metabolite secreted by several fungal species and contributes to the virulence of the human fungal pathogen *Aspergillus fumigatus*. The biosynthetic, regulatory and self-protection genes associated with GT metabolism are grouped in a 13 gene cluster. GT is an epipolythiodioxopiperazine (ETP) class fungal toxin that contains a disulfide bridge, which plays a key role in determining the deleterious effects of this toxin. Modification of this rare structural motif by reduction and *S*-methylation significantly depletes the bioactivity of this metabolite. Bisdethiobis(methylthio)gliotoxin (BmGT) is the most well characterized of such GT derivatives. Recent work from our group identified an enzyme, gliotoxin *bis*-thiomethyltransferase (GtmA), which is responsible for converting dithiol-GT to BmGT. The primary role of BmGT production in *A. fumigatus* appears to be related to regulating the GT biosynthetic gene cluster rather than working in conjunction with the GT oxidoreductase, GliT, in a GT self-protection mechanism. GliA, which is a member of the major facilitator superfamily of membrane proteins, appears to be a GT-specific membrane efflux pump and BmGT production allows *A. fumigatus* to efflux this molecule in a GliA-independent manner. Given that the main intracellular methyl donor source is *S*-adenosylmethionine (SAM), which is generated via the methyl/methionine cycle and involves *S*-adenosylhomocysteine (SAH) production, the generation of BmGT provides a direct link between GT biosynthesis and primary metabolic processes. In support of this hypothesis we have identified and characterized changes that occur in SAM and SAH homeostasis within selected *gli* gene deletions, which also uncovers new findings that illuminate additional systems interactions which have evolved in gliotoxin-producing, compared to gliotoxin-naïve, fungi to facilitate its cellular presence. Our results suggest that proteins involved in gliotoxin self-protection, regulation and efflux work in concert to maintain homeostasis of important cellular metabolites.

72. Non-trichothecene sesquiterpenes are produced by *Fusarium graminearum* PH-1, and are dependent on Tri5 expression. Chris Flynn[1], Karen Broz[2], Valeriu Bortnov[1], Claudia Schmidt-Dannert[1], H. Corby Kistler[2]. 1) University of Minnesota. Department of Biochemistry, Molecular Biology, and Biophysics. Saint Paul, MN, USA; 2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

Deoxynivalenol (DON) is the primary mycotoxin produced by the cereal disease causing ascomycete *F. graminearum*. DON is a trichothecene sesquiterpene derived via the trichodiene synthase (Tri5) product trichodiene. Sesquiterpenes, produced by sesquiterpene synthases (STS), are fifteen-carbon compounds made by many plants, bacteria, and fungi, often as defensive, pathogenesis-related, or signaling compounds. Tri5 is one of eight putative STSs in the *F. graminearum* genome. Here we describe Solid-Phase MicroExtraction (SPME) sampling of the volatile and soluble fractions of *F. graminearum* PH-1 shake flask cultures. When grown in trichothecene induction medium, PH-1 produces trichothecenes as expected, as well as several unanticipated non-trichothecene sesquiterpenes, whereas no volatile terpenes were detected when grown in non-inducing medium. Surprisingly, a *Δtri5* deletion strain grown in inducing conditions not only ceased production of the anticipated trichothecenes, but also did not produce the non-trichothecene sesquiterpenes. To test the possibility that *F. graminearum* Tri5 is a non-specific STS directly producing all observed sesquiterpenes, Tri5 was cloned from *F. graminearum* PH-1 cDNA, expressed in *E. coli*, and shown to produce primarily trichodiene. Therefore, while Tri5 expression in *F. graminearum* PH-1 is necessary for non-trichothecene sesquiterpene biosynthesis, direct catalysis by Tri5 is not sufficient to explain the diversity of sesquiterpenoids produced under trichothecene inducing conditions, nor can it explain the sesquiterpene deficient phenotype observed in the *Δtri5* strain. These findings suggest that the Tri5 expression, through an as-of-yet unidentified mechanism, is required for expression of non-trichothecene producing STSs under DON inducing conditions. While the role of trichothecenes in phytotoxicity is known, the biological function of non-trichothecene sesquiterpenes, specifically co-produced with trichothecenes, has not yet been determined.

73. The mechanism of activation of patulin biosynthesis by ammonia during *Penicillium expansum* fruit colonization. Shiri Barad[1,2], Eduardo Espeso[3], Amir Sherman[4], Dov Prusky[1]. 1) Post Harvest Sci, Agricultural Res Org, Bet Dagan, Israel; 2) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 3) Department of Molecular and Cellular Biology, Centro de Investigaciones Biologicas (C.I.B.), Madrid, Spain; 4) Genomics Unit, ARO, The Volcani Center, Bet Dagan 50250, Israel.

Penicillium expansum, the causal agent of blue mold rot, causes severe postharvest fruit maceration through secretion of D-gluconic acid (GLA) and secondary metabolites such as the mycotoxin patulin in colonized tissue. While patulin was described to be produced by many *Penicillium* species, the conditions inducing patulin biosynthesis are not clear. Analysis of the pH modulating factors produced by *P. expansum* detected that beside the production of GLA, the fungus can accumulate ammonia, both in culture and in vivo, at the leading edge of the colonized tissue. Treatment with exogenic ammonia to growing *P. expansum* on solid media induced patulin biosynthesis by inducing the expression of the global pH regulator and the secondary-metabolism-regulating transcription factors *pacC* and *laeA*, respectively. Experiments, by which ammonia was modulated by growth under limited or excessive carbon source, showed that ammonia is an efficient inducer of the expression of intermediate factors modulating patulin accumulation, including *pacC, laeA* and *idh* (one of the last genes in patulin biosynthesis), at pH ranges of 3.7-4.0 in the presence of GLA. Analysis of relative expression of *pacC* at acidic buffered conditions indicated that ammonia may induce a 2 fold increase in the relative expression of *pacC* at pH 4.5, probably as a result of internal fungal alkalization. Present result indicates that conditions enhancing ammonia accumulation by colonizing *P. expansum* may activate patulin accumulation and several pathogenicity factors which contribute to host-tissue colonization by *P. expansum* under acidic conditions.

74. Amanitin macrocyclization catalyzed by a dedicated prolyl oligopeptidase from *Galerina marginata*. Jonathan Walton[1], Sung-Yong Hong[1], Hong Luo[1], R Michael Sgambelluri[1], Evan Angelos[1], Xuan Li[2], Joshua Herr[3]. 1) DOE - Plant Research Lab, Michigan State University, East Lansing, MI; 2) Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650091, Yunnan, People's Republic of China; 3) Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing MI.

The cyclic peptide toxins of deadly poisonous mushrooms, including the octapeptide α-amanitin and the heptapeptide phalloidin, are biosynthesized on ribosomes as 35 (*AMA1*) or 34 (*PHA1*) amino acid propeptides, respectively. Recent genome sequencing of *Amanita phalloides* and *A. bisporigera* indicate that these two fungi have 30-35 related genes in the "MSDIN" family, with only a few of them in common. The regions of the AMA1 and PHA1 propeptides that appear in the final mature toxins are flanked by Pro residues, implicating a Pro-specific oligopeptidase in the first post-translational processing step. By transformation-mediated gene disruption in the amanitin-producing mushroom *Galerina marginata*, we show that a specific prolyl oligopeptidase (GmPOPB) is required for amanitin biosynthesis. Most mushroom genomes have one "housekeeping" POP gene (POPA), whereas amanitin-producing species of *Amanita* and *Galerina* have two (POPA and POPB). Pure GmPOPB obtained by recombinant expression in yeast catalyzes two reactions in a nonprocessive manner: hydrolysis at the upstream flanking Pro to release a 25mer intermediate from the 35mer propeptide, and transpeptidation at the second Pro to produce the cyclic octamer, cyclo(IWGIGCNP). The k_{cat} for cyclization of the 35mer and 25mer are 5.6 sec^{-1} and 5.7 sec^{-1}, respectively, comparable to the most active known peptide macrocyclase. Cyclization by GmPOPB required a terminal Cys and a C-terminal α-helix secondary structure in the propeptide, but not the N-terminal 10 amino acids. GmPOPB also cyclized the 35mer α-amanitin propeptide from *A. bisporigera* despite only 34% identity to the *G. marginata* in the flanking sequences. GmPOPA appears to be a conventional POP because it does not proteolytically cleave a peptide as large as 35 amino acids and does not catalyze macrocyclization.

75. A synthetic biology approach for unveiling the ecologically-relevant secondary metabolites in phytopathogens. Yit-Heng Chooi, Peter Solomon. Research School of Biology, Australian National University, Acton, ACT, Australia.

Secondary metabolites (SMs) are known to play important roles in the virulence and adaptation of fungal phytopathogens. The genome of the major wheat pathogen, *Parastagonospora nodorum*, harbors 38 SM gene clusters. An ecological genomics approach was used to

narrow down candidate SM gene clusters of potential pathogenic or ecological importance. A combination of reverse genetics and synthetic biology approaches were used to tease out the molecules encoded by these candidate clusters. Among them is a *P. nodorum* polyketide synthase (PKS) gene (*SN477*) that was highly expressed during wheat leaf infection. Heterologous expression in yeast demonstrated that *SN477* encodes the production of (*R*)-mellein. Whilst mutants of *P. nodorum* unable to synthesize (R)-mellein remained pathogenic, exposure of the compound to wheat seeds inhibited the germination. Another *P. nodorum* SM cluster harboring the PKS gene *SN8614* was up-regulated during the transition from necrotrophy to saprotrophy stage *in planta*. The overexpression of a transcriptional factor in the gene cluster activated the expression of this pathway in culture medium producing a red pigment not found in wild-type. This pigment caused significant necrosis on wheat leaves in a light-dependent manner. The compound structure was established to be a perylenequinone named elsinochrome C. This is the first report of elsinochrome C in *P. nodorum*. . We are currently reconstructing the whole pathway to understand the biosynthesis of this light-activated toxin. Finally, we were able to identify the PKS gene (*SN15829*) that encodes the production of the mycotoxin alternariol in *P. nodorum.* This was confirmed by both reverse genetics and heterologous expression of the PKS gene in *Aspergillus nidulans*. The *P. nodorum* alternariol PKS gene is different from that reported in *Alternaria alternata* previously. The identification of *P. nodorum* alternariol PKS gene will facilitate the detection of alternariol-producing fungi in the environment. In conclusion, synthetic biology can be a promising tool for linking ecological genomics with chemical ecology.

76. Production of metabolically engineered biosurfactants from basidiomycetes. Hans-Tobias Deinzer[1], Uwe Linne[2], Björn Sandrock[1], Michael Bölker[1]. 1) Biology, University Marburg, Marburg, Germany; 2) Chemistry, University Marburg, Marburg, Germany.

Many fungi are able to synthesize secondary metabolites. These are not essential for viability but provide advantages over other microorganisms.

One important group of secondary metabolites are glycolipids that act as biosurfactants. In general, they increase the availability of hydrophobic nutrients and enhance attachment to nonpolar surfaces. In some cases, they also display antimicrobial activity.

The basidiomycetous fungus *Ustilago maydis* produces large amounts of two structurally different extracellular glycolipids. Under conditions of nitrogen starvation *U. maydis* cells secrete Ustilagic acid (UA), a cellobiose lipid with antimicrobial activity and the extracellular oil mannosylerythritol lipid (MEL). The genes involved in the synthesis of both biosurfactants have been identified and both are clustered in the genome.

We could show that strains overexpression of a P450 monooxygenase results in the production of hydroxylated MELs. To generate other metabolically engineered biosurfactants, genes involved in MEL formation in other fungi, were expressed in *U. maydis*. We could identify novel MEL species by liquid chromatography and mass spectrometry.

Furthermore, we aim to identify unknown glycolipids from other smut fungi. We were able to identify new MEL species from *Sporisorium scitamineum* and *Macalpinomyces eriachnes*..

77. How to efficiently move a 24 kb gene cluster between fungal species. Rasmus J.N. Frandsen, Mikkel R. Nielsen, Paiman Khorsand-Jamal, Kristian F. Nielsen. Systems Biology, The Technical University of Denmark, Kgl. Lyngby, Denmark.

The genome sequencing of many fungal species has offered us a glimpse into a surprisingly large unutilized potential for production of novel bioactive compounds. Unfortunately, many of these compounds remain out of reach, as they are not produced under standard laboratory conditions and because we lack efficient molecular biological tools for the majority of the sequenced species. A frustrating situation, than can either be solved by developing molecular genetic tools for the individual species; Or by transferring the putative secondary metabolite gene clusters to model species, where the tools have already been established. The latter solution requires that large DNA fragments can be transferred efficiently into the model fungus, a problem that classically has been solved using time-consuming BAC/COSMID strategies. In an effort to better this situation, we set out to develop a molecular genetic tool that would allow for the efficient and fast transfer of large DNA fragments into *Aspergillus nidulans*. The fully developed system depends on a single *E. coli* based cloning reaction, to generate a targeting/assembly cassette that allows for targeted integration of the heterologous DNA into the recipient's genome. The remaining parts of the DNA sequence to be transferred are then PCR-amplified in 8-10 kb fragments (1-1½ overlap) using a high fidelity DNA polymerase. Following purification, the overlapping fragments and targeting cassette were used to transform *A. nidulans*, with assembly *in vivo* via homologous recombination. The system was tested by transferring the 24 kb aurofusarin (pigment) gene cluster from *Fusarium graminearum* into *A. nidulans*. Analysis of the resulting transformants, using diagnostic PCR, showed that all fragments had assembled as expected, thereby proving that *in vivo* multi fragment assembly is possible in *Aspergillus*. Chemical analysis of the obtained transformants showed that the constructed strains produced the expected metabolites. Subsequent overexpression of the cluster specific transcription factor (*aurR1*) boosted production of aurofusarin.

78. Overexpression of the *laeA* gene leads to increased production of cyclopiazonic acid in *Aspergillus fumisynnematus*. Inhyung Lee, Eun Jin Hong, Na Kyeong Kim, Doyup Lee. Bio and Fermentation Convergence Technology, Kookmin Univ, Seoul, South Korea.

Many of the secondary metabolite (SM) gene clusters are known to be silent in lab culture conditions. To activate these silent SM gene clusters, the *laeA* gene that encodes a global positive regulator of SM gene cluster is being used in several filamentous fungi. In addition, this approach has been also used to explore novel bioactive compounds and to improve strains used for functional food production. In this study, the overexpression of the *laeA* gene under the *alcA* promoter resulted in the production of less pigments along with shorter conidial head chains and less conidia in *Aspergillus fumisynnematus* F746. TLC and HPLC analysis revealed that SM production in *OE::laeA* was increased significantly with putative new metabolites that were not detected in wild type. Among them, a compound named F1 was

selected on the basis of the production levels and antibacterial effects. F1 was purified by column chromatography and preparative thin layer chromatography (TLC) and then identified to be cyclopiazonic acid (CPA) that was previously known as mycotoxin by LC/MS. These results suggest that overexpression of the *laeA* gene may increase the production of not only useful bioactive compounds but also toxic compounds like mycotoxins. Therefore, the strategy for the strain improvement for healthy functional food manufacturing by *laeA* overexpression should be addressed with caution.

79. Characterization of a new secondary metabolite gene cluster in *Claviceps purpurea*. Lisa Neubauer, Julian Dopstadt, Hans-Ulrich Humpf, Paul Tudzynski. University of Münster, Germany.

The phytopathogenic ascomycete *Claviceps purpurea* is an important food contaminant as it infects a broad range of grasses including economically important cereal crop plants. The best characterized secondary metabolites of *C. purpurea* are the toxic ergot alkaloids produced in the sclerotia, the survival structure of the fungus. Apart from that, little is known about the secondary metabolism of *C. purpurea* and not all toxic substances going along with the food contamination with *Claviceps* are known yet.

The availability of the *C. purpurea* genome sequence allowed a bioinformatical screening approach for typical secondary metabolite key enzymes. The presence of 9 polyketide synthases and 18 nonribosomal peptide synthetases shows the great potential of *C. purpurea* for producing secondary metabolites[1].

We were able to identify a so far unknown gene cluster in *C. purpurea* which shows high homology to the sirodesmin cluster in *Leptosphaeria maculans*[2] and the gliotoxin cluster in *Aspergillus fumigatus*[3]. Sirodesmin and gliotoxin are members of the class of epipolythiodioxopiperazine (ETP) toxins characterized by the presence of an internal disulfide bridge.

By overexpressing the cluster-specific transcription factor we were able to activate the gene cluster in *C. purpurea* and mass spectrometry analyses show different peak profiles for the overexpression mutants in comparison to the wild type. Structure elucidation of these compounds by NMR spectroscopy is ongoing, but first results confirm the bioinformatical prediction that this gene cluster is responsible for the formation of a so far unknown ETP.

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[2] Gardiner DM, Cozijnsen AJ, Wilson LM, Pedras MSC, Howlett BJ. (2004) Mol. Microbiol. 53: 1307–1318
[3] Gardiner DM and Howlett BJ. (2005) FEMS Microbiol Lett. 248: 241-248.

80. Secondary metabolite with antimicrobial activity synthesized by an edible smut fungus, *Ustilago esculenta* - from genomics and biochemistry. Cheng Ou-Yang[1], Tzong-Huei Lee[2], Wei-Chiang Shen[1]. 1) Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, Taiwan; 2) Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan, Taiwan.

The smut fungus *Ustilago esculenta* Hennng can parasitize on *Zizania latifolia* Turcz., one kind of wild rice species, to cause smut disease. The infection leads to produce swollen tissues at host stem base; however, the swollen galls are edible and have been a favorable vegetable crop, called Jiaobai in Taiwan, in Asia regions. Studies in our laboratory found that *U. esculenta* shows antimicrobial activity on certain media, suggesting that secondary metabolite(s) may be produced by *U. esculenta* for this inhibitory activity. Previous studies showed *U. maydis* and related fungal species belonging to genus *Psuedozyma* can produce some rare amphipathic glycolipids, cellobiose lipids (CLs), with antimicrobial activity. Here, we report a gene cluster may be involved in the biosynthesis pathway of CL(s) in *U. esculenta*. According to genome information, we found 11 clustered genes in *U. esculenta* genome with high similarities to genes involved in the biosynthetic pathway of ustilagic acids, CLs produced by *U. maydis*. Besides physical co-localization in the genome, consensus motifs in the promoter regions of all these genes were identified, suggesting the cluster may be transcriptionally co-regulated. Under induction conditions, these genes were apparently induced and the putative needle-like CL crystals were seen in the *U. esculenta* culture. Metabolites from *U. esculenta* culture were extracted and subjected to chemical analysis to elucidate their structures. Through the combination of genetic studies and chemical analyses, we expect *U. esculenta* is a novel CL producer.

81. RNA-seq analysis of *Cercospora beticola* DMI-resistant and -sensitive strains in response to tetraconazole. Melvin Bolton[1], Luigi Faino[2], Bart Thomma[2], Gary Secor[3]. 1) USDA - ARS, Fargo, ND, USA; 2) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 3) 3Department of Plant Pathology, North Dakota State University, Fargo, ND, USA.

The hemi-biotrophic fungus *Cercospora beticola* causes Cercospora leaf spot (CLS) of sugarbeet. CLS management measures rely on the application of sterol demethylation inhibitor (DMI) fungicides. Reduced sensitivity to DMIs has been reported recently in sugarbeet growing regions worldwide. We have shown previously that *CbCyp51*, which encodes the DMI target enzyme sterol P450 14α-demethylase in *C. beticola*, is over-expressed in DMI-resistant isolates. After exposure to tetraconazole, DMI-resistant isolates respond with further induction of *CbCyp51*. However, no *CbCyp51* promoter or gene mutations were associated with DMI resistance. To gain additional insight and identify other mechanisms involved with DMI-resistance, we used RNA-seq to identify genes differentially expressed in DMI-resistant and -sensitive *C. beticola* isolates upon exposure to tetraconazole or the control treatment *in vitro*. Interestingly, both the resistant and sensitive isolates responded with a marked induction of 15 of the 22 genes in the ergosterol biosynthesis pathway in response to tetraconazole. All 15 genes were induced to similar expression levels between the two isolates except for *CbCyp51* and *CbErg3*, which were ~16- and 2-fold higher in the resistant isolate when compared to the sensitive isolate, respectively. In total, 110 genes were uniquely differentially-expressed in the DMI-resistant isolate. Genes previously implicated with fungicide resistance such as ABC and MFS transporters were identified. The most highly induced gene in the DMI-resistant isolate encodes a protein with multiple transmembrane regions known to provide DMI resistance in yeast. This protein is not known to provide

efflux activity, but may bind directly to the fungicide. Further characterization of this gene and a detailed pathway analysis will be presented.

82. The cellulase specific transcriptome of *Trichoderma reesei* as influenced by light, photoreceptors and CRE1. Eva Stappler[1], Christoph Dattenböck[1], Doris Tisch[2], André Schuster[2], Monika Schmoll[1]. 1) Health & Environment, Bioresources, AIT - Austrian Institute of Technology, Tulln, Austria; 2) Institute of Chemical Engineering, TU Wien, Vienna, Austria.

Trichoderma reesei is adapted to degradation of plant cell walls and produces an efficient enzyme cocktail for this task. We investigated two regulation levels of the transcriptome of *T. reesei*: light and carbon source dependent control. We evaluated regulation by light, induction specific regulation and relevance of photoreceptors, a potential relevance of surface sensing in cellulase regulation and genes associated with repression of cellulase gene expression. We show that the carbon source (cellulose, lactose, sophorose, glucose, glycerol) is the major source of variation, with light having a modulating effect. 206 genes are significantly regulated in response to light across all carbon sources. 907 genes were specifically regulated under inducing conditions in light and 947 genes in darkness with 530 genes overlapping (1324 in total). In this gene set, significant enrichment was detected for functions in C-compound and carbohydrate metabolism, amino acid metabolism, energy and respiration. Evaluation of genomic distribution of genes regulated by light on cellulose, showed considerable overlap with previously described CAZyme clusters. Of the 1324 genes regulated specifically under inducing conditions in light, darkness or both, only 218 genes were found to be induction specific independent of light and not regulated by the photoreceptors. 282 of those 1324 genes were found to be regulated by CRE1 under inducing conditions. Comparison of gene expression upon growth on soluble (lactose, sophorose) and insoluble (cellulose) inducing carbon sources suggests the operation of a sensing mechanism for solid substrates, putatively involving G-protein coupled receptors, which regulates auxiliary proteins such as CIP1, CIP2 and swollenin as well as a hydrophobin gene. In order to evaluate the relevance of nutrient sensing for cellulase gene expression, we selected 9 GPCRs for functional analysis and 5 of them were found to be relevant for cellulase expression.

83. *Ustilago maydis* zinc transporters genes are functional orthologs of *Saccharomyces cerevisiae* ZRT1 and ZRT2. Adriana Mayrel Martha-Paz[1], David Eide[2], Elva Arechiga-Carvajal[1]. 1) Microbiology and Immunology Deparment, U. Autónoma de Nuevo León, San Nicolás de Los Garza, N. L., Mexico; 2) Nutritional Sciences Department, University of Wisconsin-Madison, Wisconsin, USA.

Ustilago maydis, the basidiomycete fungus that causes corn smut, is considered a pest by farmers in some countries, while in Mexico it is considered a culinary value. In some cases extreme ambient pH can lead to cellular mineral deficiency or toxicity because the solubility of metals such as zinc is affected by this condition. In yeast *Saccharomyces cerevisiae* two main systems for zinc acquisition were found; one of high affinity (ZRT1), that is highly expressed when intracellular concentrations of zinc are low and the other one of low affinity (ZRT2) mainly expressed in higher concentrations. In our work, we studied two ZRT homologous genes found in *U. maydis.* By protein alignment *U. maydis* ZRT1 and *S. cerevisiae* ZRT1 show 37% amino acid similarity and both present a ZIP domain. On the other hand *U. maydis* ZRT2 has 44% similarity with the *S. cerevisiae* ZRT2 protein and possess a ZIP and another metal binding domain. In order to test functional homology, a *zrt1D zrt2D S. cerevisiae* mutant was complemented with the *U. maydis* genes, and their growth at different zinc concentrations was measured. Recovery of *S. cerevisiae* mutant growth capacity in low and high zinc concentration media and protein sequence similarity of *U. maydis* genes allow us to conclude that these genes are homologous to those in *S. cerevisiae.* Additionally, gene expression analysis showed that ZRT2 expression is pH dependent in *U. maydis*. These results provide insights for understanding the mechanisms of adaptation of this important phytopathogen to variations of zinc concentration and availability influenced by ambientpH. In addition, using this fungus as study model, these findings can be extrapolated to other members of the basidiomycota phylum.

Cell Biology and Development

84. The RDS1 is required for dimorphic switching in lichen fungus *Umbilicaria muehlenbergii*. Min-Hye Jeong[1,2], Sook-Young Park[1], Jung A Kim[1], Nan-Hee Yu[1,2], Yong Hwa Cheong[3], Jae-Seoun Hur[1]. 1) Korean Lichen Institute, Sunchon National University, Suncheon, Jeonnam, South Korea; 2) Dept. of Biology, Sunchon National University, Suncheon, Jeonnam, South Korea; 3) Dept. of Bio-Environmental Science, Sunchon National University, Suncheon 540-950, Korea.

Lichen is symbiotic organism between a fungus (as mycobiont) and an alga and a cyanobacterium or both (as photobiont). It is generally known that axenically cultured lichen-forming fungi grow extremely slowly as hyphal form. Unlike other lichen-forming fungi, dimorphic lichen fungus, *Umbilicaria muehlenbergii*, grown easily and quickly as yeast-form cells in liquid nutrient media. This observation allows to establishing the first successful transformation system for lichen fungus. Here we report a gene, *RDS1* (Regulation of fungal Dimorphic Switch 1), which controls the dimorphic switching between yeast form and hyphal form of *U. muehlenbergii.* In the previous study, we developed and generated random insertional transformants using yeast-form cells of *U. muehlenbergii* via the use of *Agrobacterium tumefaciens*. Over 1,000 transformants have been created. During screening of the mutants, varied phenotypes were found in their color, growth and morphologies compared to wild-type. Interestingly, a hyphal form growing mutant, Um-270, was isolated. To identify T-DNA insertion region of the mutant Um-270, thermal asymmetric interlaced polymerase chain reaction was employed and identified as a T-DNA insert in upstream region of a putative C2H2-type zinc finger transcription factor *RDS1*. Light microscopic and scanning electron microscopic observation clearly showed that the mutant Um-270 apparently represents hyphal form, suggesting the responsible gene, *RDS1*, might be essential for dimorphic switching of *U. muehlenbergii*. RNA-seq analysis was used to identify several genes whose transcription during mycelial growing depends on *RDS1*. RNA-seq analysis also revealed several *RDS1*-dependent genes which may encode various factors determining cell cycle-related genes. More detail information will be described.

85. Role(s) of a chitin binding mucinoid protein, Cbp1, in appressorium differentiation of *Pyricularia oryzae*. Misa Kuroki[1], Ken-ichiro Saitoh[2], Tohru Teraoka[2], Megumi Narukawa[1], Takashi Kamakura[1]. 1) Tokyo University of Science, Japan; 2) Tokyo University of Agriculture and Technology, Japan.

Fungal pathogen, *Pyricularia oryzae*, causes serious damages to rice cultivation and influence the rice harvest all over the world. *P. oryzae* differentiates infection specific structure, known as appressoria, at the tip of germ tubes and invade to host cell. To form appressoria, *P. oryzae* seems to perceive environmental inducers such as hydrophobicity, hardness and cutin monomers. However, it remains unexplained how these signals involved in forming appressoria. Thus, revealing infection mechanism of *P. oryzae* contributes to counteract the damage of rice diseases. In addition, we can make *P. oryzae* differentiate appressoria on artificial substance under proper laboratory conditions and observe phenotypical change by adding chemicals. Formation of appressoria of *P. oryzae* can be a model of eukaryotic cellular differentiation. We focused on *Chitin Binding Protein1* (*CBP1)* that is estimated to have a role in forming appressorium. *CBP1* is specifically expressed in the germling and *cbp1* null mutants delayed in appressorium formation on hydrophobic artificial substrate. However, the reduced ability to form appressorium was restored by adding of appressorium forming inducer such as 1,16-hexadecanediol or 3-isobutyl-1-methylxanthine. These results suggested that Cbp1 plays an important role in infection signal pathway. We performed appressorium formation assay under various conditions in order to reveal which signal pathway Cbp1 is involved in. Furthermore, we generated *cbp1msb2* double mutant strains from Japanese isolate P2 and observed the appressorium formation under various conditions too. *P. oryzae* has only two mucin proteins; Msb2 and Cbp1, so, these data help us to understand Cbp1's function*. We also tried to confirm whether Cbp1 works in signal pathways as a chitin-deacetylase (CDA) or not, because Cbp1 has homology to CDA. We found that the mutants of CDA-active-site in Cbp1 failed to form appressorium on the hydrophobic surface. *Further characterization of surface recognition mechanisms in *Magnaporthe oryzae*. G. Wang *et al.*, 2013, Fungal Genetics.

86. Transcriptome analysis and phenotypic comparison between knockout and knockdown mutants of *Ehs1* in *Pyricularia oryzae*. Kana Okauchi[1], Keina Murano[1], Takumi Hirosawa[1], Megumi Narukawa[1], Takayuki Arazoe[2], Yoshikazu Arai[2], Jun Ohgane[2], Shigeru Kuwata[2], Takashi Kamakura[1]. 1) Dept. of Applied Biological Sci., Tokyo Univ. of Science; 2) Dept. of Agriculture, Meiji Univ.

Pyricularia oryzae, a pathogenic filamentous fungus, causes rice blast disease considered to be one of the most serious diseases of cultivated rice. *P. oryzae* conidium germinates on the surface of the host plant, and then, it forms a dome-shaped infection specific structure at the edge of a germ tube. This structure is called the appressorium. The matured appressorium is necessary for infection. Previous studies have shown that under appressorium forming condition, calcium ion levels rise at the site of germ tube emergence, at the tip of elongating germ tube, and in the developing appressorium. In addition to those findings, it has also been reported that Ca^{2+} channel inhibitors ($GdCl_3$ and $LaCl_3$) decrease the appressorium formation rate concentration dependently.

We aimed to elucidate the role of Ca^{2+} channels in appressorium formation. In particular, we focused on the *Ehs1* (for echinocandin hypersensitive 1) gene. This gene is considered to be a homolog of *Saccharomyces cerevisiae MID1* (for mating-induced death 1) gene encoding a stretch activated permeable Ca^{2+} channel. It is reported that the *Ehs1* knockdown mutant of *P. oryzae* Br48 strain displayed the decrease in the rates of sporulation and appressorium formation (Nguyen, *et al*., 2008). In our study, we also produced knockdown and knockout mutants. The *Ehs1* knockdown mutants of *P. oryzae* MK2-2 strain, derived from P2 (the Japanese isolate) strain, showed the decrease similar to Nguyen, *et al*., 2008, while the *ehs1* deletion mutants of P2 strain did not display significant decrease compared to the controls.

Therefore, in order to determine which is the more authentic mutant reflecting the phenotype of *ehs1* deletion mutant truly, we conducted a comparative transcriptome analysis between the knockout and knockdown mutants by the next-generation sequencing technologies.

87. Oxygen and an Extracellular Phase Transition Independently Control Central Regulatory Genes and Conidiogenesis in *Aspergillus fumigatus*. Myoung-Hwan Chi, Kelly Craven. Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

Conidiogenesis is the primary process for asexual reproduction in filamentous fungi. As the spores, or conidia, resulting from the conidiogenesis process are primarily disseminated via air currents and/or water, an outstanding question has been how fungi recognize aerial environments suitable for conidial development. In this study, we documented the somewhat complex development of the conidia-bearing structures, termed conidiophores, from several *Aspergillus* species in a subsurface layer of solid media. Conidiophores are typically comprised of stalks, vesicles, phialides and conidia, and although these *Aspergillus* species were able to develop conidiophores in gel-phase environment, exposure to the aeriform environment was required for the terminal developmental transition from phialide cells to conidia. Phialides confined to a gel-embedded environment were unable to differentiate into conidia and instead, formed secondary conidiophores or apically-elongated phialides. Our observations of conidiophore development in high or low oxygen conditions in both aeriform and gel-phase environments revealed that oxygen and the aeriform state are positive environmental cues for inducing conidiogenesis in most of the aspergilli tested in this study. Transcriptional analysis using *A. fumigatus* strain AF293 confined to either the aeriform or gel-phase environments revealed that expression of a key regulatory gene for conidiophore development (*AfubrlA*) is facilitated by oxygen while expression of another regulatory gene controlling conidia formation from phialides (*AfuabaA*) was repressed regardless of oxygen levels in the gel-embedded environment. Our findings provide not only novel insight into how fungi recognize an aerial environment to trigger the developmental shift for airborne conidia production but also the relationship between environmental cues and conidiogenesis regulation inaspergilli.

88. The *Aspergillus fumigatus* farnesyltransferase β-subunit, Ram1, regulates Ras protein localization, conidial viability and antifungal susceptibility. Tiffany S. Norton, Rachel V. Lovingood, Qusai Al Abdallah, Jarrod R. Fortwendel. University of South Alabama, Mobile, AL.

Post-translational prenylation mechanisms, including farnesylation and geranylgeranylation, mediate both subcellular localization and protein-protein interaction in eukaryotes. The farnesyltransferase (FT) enzyme complex is composed of two subunits: the α-subunit, an essential protein shared with the geranylgeranyltransferase complex; and a β-subunit, termed Ram1. FT activity is an important mediator of Ras pathway signaling via control of Ras protein localization. Our previous data show that *A. fumigatus* RasA localizes primarily to the plasma membrane where it functions in processes controlling morphogenesis and virulence. However, the importance of FT activity to Ras protein function, filamentous fungal growth, and *A. fumigatus* virulence is currently unknown. To explore this, we generated an *A. fumigatus* deletion mutant lacking the FT β-subunit (Δ*ram1*). Conidial germination rate was reduced in the Δ*ram1* mutant, with a concomitant reduction in conidial viability of 45%. Although no polarity defects of hyphae were apparent, the Δ*ram1* mutant displayed reduced radial growth rate, an average increase in hyphal width of 26%, and altered nuclear positioning in growing hyphae. Furthermore, loss of *ram1* resulted in resistance to triazole antifungal drugs such as voriconazole. Complementation of the Δ*ram1* mutant with the *ram1* gene (Δ*ram1+ram1*) restored the wild-type phenotype for each of these processes. To define molecular mechanisms for Ram1-mediated processes, we generated strains expressing GFP-RasA in the Δ*ram1* genetic background. The absence of *ram1* resulted in mislocalization of RasA from the plasma membrane. Interestingly, mutation of RasA to enhance selectivity for geranylgeranylation as an alternative membrane targeting mechanism in the absence of Ram1 restored RasA plasma membrane localization but not radial growth. These data suggest that Ras-independent mechanisms are at least partially responsible for phenotypes exhibited by the Δ*ram1* mutant. Together, these data point to a crucial role for the Ram1 farnesyltransferase in mediating *A. fumigatus* growth and antifungal susceptibility.

89. Characterization of Myosins in *Aspergillus fumigatus* Growth and Pathogenesis. Hilary Renshaw[1], Praveen R. Juvvadi[2], José M. Vargas-Muñiz[1], Amber D. Richards[2], William J. Steinbach[1,2]. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Pediatrics, Duke University Medical Center, Durham, NC.

Aspergillus fumigatus is the etiological agent of invasive aspergillosis, a leading cause of death in immunocompromised patients. Invasion of host tissue by this fungus is facilitated by polarized hyphal growth and septation. Myosins are a group of motor proteins known to be involved in hyphal growth, morphology and septation in fungi. However, the role of myosins in the growth and virulence of a human pathogen has never been explored. In this study, we investigated *A. fumigatus* myosins belonging to three different classes: class I (MyoA), class II (MyoB), and class V (MyoE). We generated two myosin single deletion strains (Δ*myoB* and Δ*myoE*) and a double deletant (Δ*myoB* Δ*myoE*). Attempts to delete the *myoA* gene were unsuccessful, suggesting its essential nature. While the Δ*myoB* strain showed aberrant septation it was also hypersensitivity to anti-cell wall agents revealing its role in maintaining cell wall integrity. Deletion of *myoE* resulted in reduced hyphal extension and hyperbranching, indicating its role in preserving hyphal polarization and/or suppressing new growth foci. In addition, the Δ*myoE* strain displayed hyperseptation albeit with normal septa, suggesting that MyoE is necessary for regular septation. In contrast to the Δ*myoE* strain, the Δ*myoB* Δ*myoE* strain showed broader hyphae with reduced hyphal extension and absence of septation, indicating that both MyoB and MyoE are integral for septum formation. Both Δ*myoB* and Δ*myoE* strains were hypovirulent in a screening *Galleria mellonella* model of invasive aspergillosis; however, surprisingly, only the Δ*myoB* strain displayed decreased virulence in a neutropenic murine model of infection. Taken together, these data demonstrate the crucial but distinct roles that myosins play in maintaining proper hyphal morphology and for pathogenesis of *A. fumigatus*.

90. Understanding the Role of Septins in *Aspergillus fumigatus* Growth and Pathogenesis. José M Vargas-Muñiz[1], Hilary Renshaw[1], Amber D Richards[2], Erik J Soderblom[3], M Arthur Moseley[3], Praveen R Juvvadi[2], William J Steinbach[1,2]. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Pediatrics, Duke University Medical Center, Durham, NC; 3) Duke Proteomics and Metabolomics Core Facility, Center for Genomic and Computational Biology, Duke University, Durham, NC.

Aspergillus fumigatus is the major etiology of invasive aspergillosis, a leading cause of death in immunocompromised patients. Septins are conserved GTPases involved in septation, conidiation and cell wall organization. The requirement of septins for tissue invasion and virulence has been demonstrated in the human pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans*, as well as the plant pathogen *Magnaporthe oryzae*. *Aspergillus* spp. contain five genes encoding for septins (AspA-E). We performed single, double, and triple deletions of the genes encoding for AspB, AspD, and AspE in *A. fumigatus*. This approach revealed that septins AspB, AspD, and AspE are dispensable for radial extension under normal growth conditions. While septins AspB and AspE are required for regular septation, septins AspB and AspD are required for conidiation. In addition, the normally observed conidial electron-dense outer layer is completely absent in the Δ*aspB* strain. Furthermore, the Δ*aspB* strain showed increased susceptibility to anti-cell wall agents. Unlike in the model filamentous fungus *A. nidulans*, we did not observe hyperbranching in any of the *A. fumigatus* septin deletion strains, revealing different roles for these septins in a human pathogen. Using the strain expressing AspB-GFP fusion protein, we show that AspB is phosphorylated *in vivo* at 9 residues and interacts with the other septin and cytoskeletal components during the multicellular growth stage. Strains lacking AspB exhibited hypervirulence in a *Galleria* model of invasive aspergillosis, and *ex-vivo* macrophages exposed to Δ*aspB* conidia released more TNF-α compared to the wild-type strain. However, infection with the Δ*aspB* strain in a murine model did not yield a difference in virulence. Taken together, these results point to the importance of septins in *A. fumigatus* growth and development.

91. Stage-dependent subcellualr compartmentalization of fungal melanic biosynthetic machinery. Srijana Upadhyay[1], Xinping Xu[1], David Lowry[2], Jennifer Jackson[1], Robert Roberson[2], Xiaorong Lin[1]. 1) Department of Biology, Texas A&M University, College Station, TX, 77843, USA; 2) School of Life Sciences, Arizona State University, Tempe, Arizona, 85287, USA.

Melanin, produced by living organisms in the course of hydroxylation and polymerization of organic compounds, is a universal amorphous polymer that confers coloration. In the pathogenic microorganisms, melanin is correlative with increased virulence. More

importantly, melanin provides protection to its host organism against various damaging exposures. In fungi, it is known that melanin is located on the cell wall. Paradoxically, the majority of fungal species synthesize melanin de novo through the polyketide pathway that utilizes the intracellular primary metabolites and the polyketide synthase (PKS). Thus how the negatively charged macromolecules cross the plasma membrane and get externalized as melanin granules is enigmatic. Prompted by a fortuitous discovery of an insertional mutant defective in melanin deposition in the cell wall through a forward genetic screen, we began to address those questions by systematically investigating all the melanin biosynthetic enzymes in the human fungal pathogen *Aspergillus fumigatus* and the model filamentous fungus *A. nidulans*. Our findings indicate that melanin biosynthesis is initiated in secretory endosomes and their exocytosis leads to the deposition of melanin granules in the cell wall. According the discoveries, it is suggested that a unified theme in the organization of melanin biogenesis and trafficking in this special endosomal-derived organelle (melanosomes) exists in both fungi and mammals, and secretory endosomes may play a major means for the biogenesis and trafficking of fungal polyketides, a group of natural products that are vital to modern medicine and agriculture.

92. A novel role for an SR/RRM family mRNA shuttling binding protein in cell cycle regulation in *Aspergillus nidulans*. S. L. Anglin[1], S. James[2]. 1) Dept. Biology, Millsaps College, Jackson, MS; 2) Dept. Biology, Gettysburg College, Gettysburg, PA.

SR/RRM proteins are a class of small nuclear ribonucleoproteins known to have functions in spliceosome assembly and catalysis as well as in mRNA transcription and export. Heretofore, this family of proteins has not been implicated in the regulation of cell division. We recently reported that the *Aspergillus nidulans snxA* gene encodes an ortholog of budding yeast Hrb1/Gbp2 and of human hnRNP-M, members of the SR/RRM protein family. hnRNP-M is a ubiquitous and highly expressed nuclear protein in human tissues, and is known to control alternative splicing of developmentally regulated genes. SNXA localizes to the nucleus but is excluded from the nucleolus in *A. nidulans*, paralleling the localization of hnRNP-M. The *snxA1* mutation was originally identified as an extragenic suppressor of mutations in the G2/M regulatory gene $nimX^{cdc2}$, and we have shown that it suppresses mutations in multiple components of the CDC2/CYCLINB regulatory pathway, including $nimT23^{cdc25}$ and $nimE6^{cyclinB}$ but not *nimA5* or *nimA1*. We further found that both *snxA1* and a second allele, *snxA2*, are hypomorphic and that both mutations result in significantly decreased *snxA* transcription and SNXA protein levels. Additionally, mutation or deletion of *snxA* alters $NIME^{CYCLINB}$ localization patterns. Our data suggest that SNXA may normally function to restrain the G2/M transition by affecting the CDC2/CYCLINB regulatory pathway, suggesting a novel function for the SR/RRM family that links RNA metabolism and transport to regulated cell division.

93. Global examination of the molecular roles, localizations and interactomes of F-box proteins in fungal development. Oezguer Bayram[1], Oezlem Sarikaya Bayram[1], Sabine Reen[2], Gustavo Goldman[3], Gerhard Braus[2]. 1) Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland; 2) Department of Molecular Microbiology and Genetics, Georg-August University, Göttingen, Germany; 3) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil.

Multiprotein complex, Skp-Cul-Fbox (SCF) E3 ubiquitin ligases, are the largest family of E3 ligases that are responsible for marking of target proteins with ubiquitin and subsequent proteasome-dependent degradation. SCF E3 ligases are involved in many cellular processes, including transcription, cell-cycle control by determining protein levels of target proteins. The F-box component of the SCF complex is essential for the substrate specificity of the SCF complex by recruiting target proteins for ubiuitination. In this study, we have systematically investigated the molecular functions of 73 F-box or F-box-like protein encoding genes of the eukaryotic model system *Aspergillus nidulans*. Deletion of 73 *fbx* genes revealed that only 8-10 % of the *fbx* genes are required for proper fungal development and light response. Only *fbx25*, which encodes SconB necessary for sulphur metabolism, is essential for fungal growth and survival. 50 % of the F-box proteins (30-35) are associated with the SCF complexes through the adaptor SkpA protein during fungal development. Several F-box proteins show development and stress specific interactions with the SkpA protein. 30 % of the F-box proteins are exclusively localized to the nuclear fraction whereas the rest show other localization patterns including, cytoplasmic, hyphal tip and plasma membrane. High scoring F-box proteins (Fbx1 to Fbx48) interact with more than 1500 proteins including SkpA and CullinA. These data suggest that F-box proteins interact with at least 15% of the total proteome and control developmental responses to environmental stimuli and stresses.

94. The role of septin AspD in *Aspergillus nidulans*. I. Dörter, M. Momany. Plant Biology Dept, UGA, Athens, GA.

Septins are evolutionarily conserved GTP-binding proteins from the GTPase superfamily that form filaments, which play roles as important and diverse as those of actin and microtubules. These new and increasingly characterized cytoskeletal components act as diffusion barriers and coordinate cytokinesis and nuclear division.

Septins are classified into five orthologous groups, and monomers from different groups associate to form nonpolar heteropolymeric rods, which in turn assemble into higher-order structures including rings and filaments that can be visualized by fluorescent microscopy of GFP-tagged septins. The mechanisms driving septin heteropolymer and higher-order structure assembly are only beginning to be understood.

The filamentous fungus *Aspergillus nidulans* has one septin from each phylogenetic group. Four of the *A. nidulans* septins are orthologs of the core septins in *S. cerevisiae* and the fifth septin, AspE, is lacking in unicellular yeasts and appears to be ancestral. Our results show that at least two distinct septin heteropolymer populations co-exist in *A. nidulans*.

According to our data, the septin AspD, which is homologous to *Saccharomyces cerevisiae* Cdc10p, is only present in one of the heteropolymers. In contrast to the other three core septins, deletion of AspD did not show emergence of extra germ tubes and branches in early development, but *ΔaspD* deletion strains revealed abnormal nuclear morphology. Further, *ΔaspD* colonies showed an increased frequency of sectoring relative to wildtype. Fluorescence microscopy and time-lapse analyses revealed AspD-GFP tagged septin rods contact nuclei and the cell cortex. Thus our results suggest that AspD might play a role in coupling nuclear division to cytokinesis.

95. Functional domains of the developmental regulator FlbB mediate the tip-to-nucleus communication in *Aspergillus nidulans* vegetative hyphae. Erika Herrero-García[2], Elixabet Perez de Naclares-Arregui[1], Marc S. Cortese[1], Ane Markina-Iñarrairaegui[1], Oier Etxebeste[1], Eduardo A. Espeso[2], Unai O. Ugalde[1]. 1) Biochemistry II Lab., Dept. of Applied Chemistry, The University of The Basque Country, 20018, San Sebastian, Gipuzkoa, Spain; 2) Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain.

Polar cells have developed multiple mechanisms to convey environmental signals from the polarity site to the nucleus and induce the appropriate cellular response. These mechanisms include transcription factors located at the polarity site, such as FlbB, which signals asexual development in vegetative hyphae of the filamentous fungus *Aspergillus nidulans*. FlbB is detected at the tip and apical (but not distal) nuclei, and understanding the relationship between these pools is crucial for the elucidation of the mechanisms that induce conidiation. Photo-convertible tagging with Dendra2 demonstrated a directionality of FlbB movement from the tip to nuclei, in a process that required an N-terminally located nuclear localization signal. Tip localization of a constitutively expressed GFP::FlbB chimera was abolished in the null mutant of its apical interactor FlbE, while the nuclear pool was increased. The aconidial phenotype of this strain demonstrated that tip processing of FlbB is a prerequisite for the induction of conidiation in nuclei. The bZIP domain of FlbB is essential and sufficient to enable the interaction with FlbE. However, the retention of FlbB at the tip also requires the C-terminal domain, since the substitution of the cysteine 382 by an alanine disrupted the apical localization. Overall, these findings demonstrate that fungal-specific adaptors and the establishment of a specific three dimensional conformation are key requirements for the apical localization of FlbB and demonstrate that nuclei are asymmetrically fed with a transcriptionally active pool originating at the tip.

96. The velvet family of fungal regulators contains a DNA-binding domain structurally similar to NF-κB. Jennifer Gerke[1], Yasar Luqman Ahmed[2], Hee-Soo Park[3], Özgür Bayram[1], Piotr Neumann[2], Min Ni[3], Achim Dickmanns[2], Sun Chang Kim[4], Jae-Hyuk Yu[3], Ralf Ficner[2], Gerhard Braus[1]. 1) Molecular Microbiology and Genetics, Georg-August University, Göttingen, Germany; 2) Molecular Structural Biology, Georg-August University, Göttingen, Germany; 3) Bacteriology and Genetics, University of Wisconsin-Madison, Madison, Wisconsin, USA; 4) Biological Sciences, Korea Advanced Institute of Science and Technology, Dae-Jon, Republic of Korea.

Fungi produce small bioactive molecules (secondary metabolites) for signaling and protection, whereas in animals the inflammation and immune system is responsible for self-defense. The regulation of secondary metabolism and the control of development in fungi are coordinated by a family of velvet-domain containing regulators, the velvet proteins. These are conserved across the fungal kingdom and share a homologous region of about 150 amino acids that lacks sequence homology to any other known protein. *In vivo* chromatin immuno-precipitation (ChIP) and *in vitro* electrophoretic mobility shift assays (EMSAs) showed that the velvet-domain of the *Aspergillus nidulans* protein VosA is a novel DNA-binding domain that specifically recognizes an 11-nucleotide consensus sequence in the promoter regions of key developmental regulatory genes. The crystal structure analysis of the VosA velvet-domain revealed an unforeseen structural similarity with the Rel homology domain (RHD) of the mammalian transcription factor NF-κB. RHD-containing proteins control inflammation, the immune system and development in animals. We identified several conserved amino acid residues in the velvet-domain and showed that they are essential for the DNA-binding ability of VosA. Additionally, we demonstrate by crystal structure analyses of the VosA homodimer and the VosA-VelB heterodimer that the velvet-domain is also important for dimer formation. These findings indicate that the coordination of development and defense mechanisms in fungi and animals might be controlled by the structurally related RHD and velvet proteins, and that they might have a common functional origin. The different homo- and heterodimers of velvet proteins might modulate gene expression of developmental and defensive pathways similar to NF-κB.

97. The *sepG* gene in *Aspergillus nidulans* encodes an IQGAP homologue. Terry Hill[1], Loretta Jackson-Hayes[2], Kristen Wendt[1]. 1) Dept Biol, Rhodes College, Memphis, TN; 2) Dept. Chem, Rhodes College, Memphis, TN.

The study of septation in filamentous fungi received a significant boost twenty years ago through the identification and characterization of several temperature-sensitive septation (*sep*) mutants in *Aspergillus nidulans* by Steven Harris and colleagues (Harris et al., 1994, Genetics 136: 517-532). One continuing loose end from that study has been the identity of the mutation designated *sepG1*. Through meiotic mapping of *sepG1*'s position on Chromosome II along with sequencing of candidate genes in the indicated chromosomal region, we have identified *sepG* as *Aspergillus nidulans* gene AN9463. The predicted gene product shows 35% identity and 55% similarity to the *Schizosaccharomyces pombe* Rng2 protein, described as an IQGAP homologue involved in septation. The mutation in *sepG1* is a G-to-A transition at position 5082 of the 5333-nucleotide open reading frame, predicted to cause a glycine-to-arginine substitution at residue 1637 of the 1737-amino acid product. The *sepG1* phenotype is complemented by the cloned wild type allele. The *N*-terminal GFP-tagged product of wild type AN9463 (GFP::SepG) colocalizes with actin and myosin during cell division, beginning with the "actin/myosin tangle" phase that precedes mature contractile ring formation, and colocalization continues through ring contraction and dissipation. GFP::SepG localization at septation sites is blocked by the *sepH1* and *sepA1* mutations. The *sepD5* mutation does not prevent localization of GFP::SepG in peripheral rings, but it does block ring constriction. Reduced expression of wild type AN9463 under the regulatable *AlcA* promoter blocks septum formation and the localization of a number of GFP-tagged septal ring-associated proteins such as AspB and the *A. nidulans* homologue of *S. cerevisiae* Cdc14. However, in a strain expressing both C-terminal tagged MyoB::GFP and the *in vivo* actin-labeling probe Lifeact::mRFP, down-regulation of AN9463 did not prevent actin or myosin from colocalizing as a ring at putative septation sites.

98. Golgi-localized and the palmitoyl transferase-related AkrA homologs mediates $[Ca^{2+}]_i$ transient to response ER and azole stresses. Yuanwei Zhang[1], Qingqing Zhen[1], Jinxing Song[1], Lina Gao[1], Alberto Muñoz[2], Nick D. Read[2], Ling Lu[1]. 1) College of Life Science, Nanjing Normal Univeristy, Nanjing, jiangsu, China; 2) Manchester Fungal Infection Group, Institute of Inflammation and Repair, CTF Building, University of Manchester, Manchester M13 9NT, UK.

Finely tuned $[Ca^{2+}]_i$ changes mediate several intracellular functions, resulting in subsequent activation or inactivation of a series of

conserved Ca^{2+} signaling components and their target proteins. Palmitoylation is a reversible post-translational modification involved in membrane protein trafficking and functional modulation. However, studies on the relationship between calcium signaling and palmitoylation have been limited. Here, we demonstrate that the homologs of yeast palmitoyl transferase ScAkr1p, AkrA in *Aspergillus nidulans* and SidR in *Aspergillus fumigatus*, play important roles under low calcium conditions. Deletion of *akrA* or *sidR* shows remarkable defects in hyphal growth and conidiation, but adding extracellular calcium can completely rescue the growth defects. Moreover, using the calcium probe aequorin in live cells, we found that all of the palmitoyl transferase-related *akrA* mutants induced larger decreases in the $[Ca^{2+}]_i$ response to extracellular Ca^{2+} compared to the previously identified high-affinity calcium influx system members (CchA and MidA) and compared to the parent control strain. Moreover, ER stressors- or azole-induced calcium transient was completely blocked by AkrA defects, especially in low calcium conditions where we did not detect a calcium transient. Interestingly, all of the above-described functions AkrA are tightly related to cysteine residues in its DHHC-CRD and its palmitoyl transferase activity. Thus, Golgi-localized AkrA mediates the $[Ca^{2+}]_i$ transient likely by globally palmitoylating calcium signaling components and their target proteins. Our findings provide insight into a new link between calcium signaling and palmitoylation in the regulation of cell survival processes upon ER and membrane stress.

99. The functional orthologue of the human tumor suppressor APC protein MigA plays a role in polarity determination in the filamentous fungus *Aspergillus nidulans*. Raphael Manck[1], Yuji Ishitsuka[2], Satur Herrero[1], Norio Takeshita[1], G. Ulrich Nienhaus[2], Reinhard Fischer[1]. 1) IAB - Microbiology, KIT, Karlsruhe, Germany; 2) CFN, KIT, Karlsruhe, Germany.

Polarity establishment and maintenance is an essential process conserved in all kingdoms and very obvious in filamentous fungi like *Aspergillus nidulans*. The cell needs an orchestrated polarization machinery to initiate and sustain a highly polarized structure such as a hypha. The microtubule (MT) and the actin cytoskeleton along with MT associated proteins such as MT plus-end tracking proteins (+TIP) play key roles in defining an internal polarity axis. In addition, MT's define the site of actin polymerization through the delivery of cell end marker proteins (1).

Here we describe MigA from *A. nidulans*, which is the first functional orthologue of the human tumor suppressor APC in filamentous fungi. APC is an essential regulator of radial glial polarity and construction of the cerebral cortex in mice (2). Furthermore it regulates axon arborization and cytoskeleton organization. MigA interacts with the membrane associated ApsA protein and is involved in spindle positioning during mitosis. Since MigA is related to yeast Kar9, this function is conserved in comparison to *Saccharomyces cerevisiae*. Moreover, MigA is also associated with septal and nuclear MT organizing centers and localizes in an EbA-dependent manner to assembling and retracting MT plus-ends. This characteristic classifies MigA as a +TIP. *A. nidulans* MigA forms a homodimer and is able to bind filamentous α-tubulin autonomously. In contrast to Kar9, MigA has another unexpected function. It is required for MT convergence and correct localization of the cell end markers TeaR and TeaA at the hyphal tip. MigA also interacts with the class V myosin MyoV. Taken together we propose an active Actin-MyoV-MigA-dependent guidance mechanism, the MigA-pathway, of MT's in the hyphal tip. Hence, actin and MT organization depend on each other.

(1) Fischer *et al.* (2008). Mol Microbiol. May; **68**(4):813-26

(2) Yokota *et al.* (2009). *Neuron*. Jan 15;**61**(1):42-56.

100. Cytoplasmic dynein is required for the coalescence and clearance of protein aggregates in filamentous fungi. Mark McClintock[1], Martin Egan[1], Ian Hollyer[1], Hunter Elliott[2], Samara Reck-Peterson[1]. 1) CELL BIOLOGY, HARVARD MEDICAL SCHOOL, BOSTON, MA; 2) IMAGE AND DATA ANALYSIS CORE, HARVARD MEDICAL SCHOOL, BOSTON, MA.

Eukaryotes have evolved multiple strategies for maintaining cellular protein homeostasis. One such mechanism involves neutralization of deleterious protein aggregates via their defined spatial segregation. Here, using the molecular disaggregase Hsp104 as a marker for protein aggregation, we describe the spatial and temporal dynamics of protein aggregates in the filamentous fungus Aspergillus nidulans. Filamentous fungi, such as A. nidulans, are a diverse group of species of major health and economic importance and also serve as model systems for studying highly polarized eukaryotic cells. We find stress-induced protein aggregates require microtubules to form discrete inclusions. Coalescence and clearance of these inclusions requires the microtubule-based motor cytoplasmic dynein. Finally, we find that impaired clearance of these inclusions negatively impacts retrograde trafficking of endosomes, a conventional dynein cargo, indicating that microtubule-based transport can be overwhelmed by chronic cellular stress.

101. Insights into the nuclear transport and function of the transcription factor FlbB. Elixabet Oiartzabal[1], Aitor Garzia[2], Eduardo A. Espeso[3], Unai Ugalde[1], Oier Etxebeste[1]. 1) Dept. of Applied Chemistry, Faculty of Chemistry, University of The Basque Country. Manuel de Lardizabal 3, 20018, San Sebastian; 2) Howard Hughes Medical Institute, Laboratory of RNA Molecular Biology, Rockefeller University, 1230 York Avenue, New York, NY 10065; 3) Dept. of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu, 9, 28040, Madrid, Spain.

The UDA (Upstream Developmental Activator) pathway of *Aspergillus nidulans* transduces environmental signals and activates asexual development. FlbB is a key UDA transcription factor (TF) which is accumulated at the most apical nucleus and the tip of vegetative hyphae, through a mechanism that requires the activity of additional UDAs. Tip accumulation of FlbB is mediated by the adapter UDA FlbE. The present work has focused on the retrograde transport of FlbB from the tip to the nucleus, and its transcriptional activity there. Nuclear import of FlbB is mediated by a bipartite nuclear localization signal located at the N-terminus. It has been previously reported that, once in the nucleus, FlbB activates the expression of *flbD* (another UDA factor coding gene), and then both factors are jointly required for the induction of the first specific asexual development regulator, *brlA*. We have now found that FlbD activity is essential for the nuclear accumulation of FlbB. FlbD and FlbB interact, and the deletion of *flbD* causes a significant reduction in the nuclear accumulation of FlbB

and an increase in its apical fluorescent intensity. Bi-directional, cytoplasmic and microtubule-dependent movement of FlbB::GFP can be easily followed in a *ΔflbD* background. RNA sequencing analyses comparing Δ*flbB* and wild-type transcriptomes show that FlbB has additional roles, such as the suppression of the *dba* metabolic cluster, responsible for the synthesis of DHMBA, an antibacterial metabolite. In addition, our study also identifies FlbB-mediated regulation of the putative Helix-Loop-Helix type TF UrdA, an inducer of asexual and repressor of sexual development. The results presented widen the scope on the transcriptional roles of FlbB and the mechanisms that allow the transduction of environmental signals from the tip to the nucleus in *A. nidulans*.

102. Conserved, rare codons encoding residues of the N-terminal region of the urea transporter UreA are necessary for proper synthesis and/or targeting to the plasma membrane. M. Sanguinetti[1], M. Veyga[1], A. Iriarte[3], S. Amillis[2], H. Musto[3], M. Marin[1], A. Ramon[1]. 1) Biochemistry Section, Dept of Cellular and Molecular Biology, Faculty of Sciences - UdelaR, Montevideo, Uruguay; 2) Faculty of Biology, Dept of Botany, University of Athens, Athens, Greece; 3) Laboratory of Genome Organization and Evolution, Dept of Ecology and Evolution, Faculty of Sciences - UdelaR, Montevideo, Uruguay.

Our group has developed an in vivo model to contribute to the understanding of how codon usage and translation kinetics can determine membrane protein folding in vivo, by making use of site-directed mutagenesis in a functional GFP-tagged version of UreA. Through an evolutionary analysis we have identified conserved "frequent" or "rare" codons in UreA orthologues from the sequenced Aspergilli. When two rare codons coding for two residues in the N-terminal portion of the protein are changed into synonymous, frequent ones, the resulting strain shows impaired ability to grow on urea at 37°C, but not at 25°C. ^{14}C-urea transport assays support these results, whereas Western blot and epifluorescence microscopy show a lower amount of protein in the membrane of the mutant strain, apparently due to a decrease in UreA synthesis or translocation to the membrane. No significant differences could be determined in the levels of mRNA or the predicted structures of the wild type and mutant strains. In order to determine differences in local translation kinetics, in vitro assays are currently being optimized. We speculate that the two conserved, rare codons could play a role in establishing a translational pause which may be important in the first steps of UreA synthesis and sorting to the membrane.

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103. Coordination of aminophospholipid asymmetry, P4 ATPases, and vesicle traffic during hyphal growth. Zachary Schultzhaus, Huijuan Yan, Brian Shaw. Plant Pathology and Microbiology, Texas A&M University, College Station, TX.

How proteins break symmetry and congregate in specific places inside cells is a major question in developmental biology, and fungal hyphae are excellent models for studying this phenomenon. Hyphae precisely localize a large group of proteins in order to colonize and maintain a constant shape. This organization includes a strict segregation of endocytosis and exocytosis into different areas of the apical compartment in growing hyphae. How endocytosis and exocytosis initialize, however, is unclear in these cells. Recently, the asymmetric nature of the phospholipid bilayer has attracted attention for playing roles in diverse processes such as phagocytosis and cell polarity. In this project we analyzed the role of phosphatidylserine distribution in fungal tip growth. The distribution of the phospholipid phosphatidylserine in growing hyphae was assessed. Phosphatidylserine concentrated on the outside of secretory vesicles, rather than the plasma membrane, in contrast to what is seen under normal conditions in budding yeast. Moreover, the deletion of phospholipid flippases caused a dispersal of phosphatidylerine secretory vesicles from the apex to the rest of the cytoplasm. These results indicate that phospholipid flippases (P4 ATPases) may be important for phosphatidylserine polarity on secretory vesicles, and thus for the localization of many tip proteins in growing cells.

104. Sclerotia formation in *Aspergillus niger* is accompanied by expression of otherwise silent secondary metabolite gene clusters. Arthur Ram, Anne-Marie Burggraaf-van Welzen, Mark Arentshorst, Thomas Jorgensen. Molecular Microbiology and Biotechnology, Leiden University, Institute Biology Leiden, Leiden, Netherlands.

Sclerotia are compact mycelial masses with hardened, thick walls and a less dense stroma. They play a role in dormancy, serving to survive adverse environmental conditions and are considered to be an important prerequisite for sexual development. Several environmental factors have been shown to be correlated to the production of sclerotia, such as absence or presence of light, medium composition, oxygen availability and temperature. *Aspergillus niger* is a biotechnologically important fungus which is only known to proliferate asexually. Sclerotia formation of naturally isolated strains of *A. niger* has only be recently reported in certain *A. niger* strains grown on specific medium containing raisins, other fruits or rice. We previously described an *Aspergillus niger* mutant (*scl-2*) displaying a reduced conidiation phenotype and forming abundant sclerotia on commonly used rich medium agar plates not supporting sclerotium formation in the WT. In this study, we characterized the sclerotia forming mutant (*scl-2*) in detail. Several lines of evidence support that the multicellular structures are indeed sclerotia: *i)* Safranin staining of microscopic coupes of the sclerotia-like structures produced showed the typical cellular structure of a cell dense outer layer and a less dense inside in the sclerotium, *ii)* formation of the sclerotia-like structures is inhibited by light iii) dependent on the oxidative state of the mycelium requiring a functional *noxA*/*noxR*-dependent NADPH oxidase complex. Genome-wide expression analysis of the *scl-2* mutant suggests specific sclerotium dependent production of indoloterpenes. Inspection of gene expression data available for *Aspergillus niger* which includes over 150 growth conditions revealed that three secondary metabolite gene clusters of which two are indicated to be related to indoloterpene synthesis were uniquely expressed in sclerotia.

105. Genome-wide transcriptome analysis of cell wall remodeling in *Aspergillus niger* in response to the absence of galactofuranose biosynthesis. Joohae Park[1], Mark Arentshorst[1], Boris Tefsen[2], Ellen Lagendijk[1], Cees van den Hondel[1], Irma van Die[2], Arthur Ram[1]. 1) Molecular Microbiology and Biotechnology, Leiden University, Institute Biology Leiden, Leiden, The Netherlands; 2) Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands.

The biosynthesis of cell wall galactofuranose (Gal*f*) containing glycostructures such as galactomannan, *N*-glycans, *O*-glycans and glycosylinositolphosphoceramides in filamentous fungi are important to secure the integrity of the cell wall. A key gene in the biosynthesis

of UDP-Galf is *ugmA* which encodes a UDP-galactopyranose mutase which is essential for the conversion of UDP-Gal*p* to UDP-Gal*f*. In A. *niger*, the absence of Gal*f* synthesis results in activation of the cell wall integrity (CWI)-pathway indicating that the Gal*f* biosynthesis is important for maintaining cell wall strength. To identify genes involved in maintaining cell wall integrity in response to the absence of galactofuranose biosynthesis, a genome-wide expression study was performed with the *ugmA* deletion strain. RNAseq analysis revealed 432 upregulated genes to be differentially expressed (Q-value <0.05) in the *ugmA* mutant compared to the wild-type and these genes encode enzymes involved in alpha-glucan synthesis (*agsA*), chitin synthesis (*gfaB, gns*A, and *chsA*), beta-glucan remodeling (*bgxA, gelF*, and *dfgC*) and several (GPI)-anchored cell wall protein encoding genes. Interestingly, also the gene encoding the CWI-specific Map-kinase-kinase (*mkkA*) was induced in the *ugmA* mutant. *In silico* analysis of the 1-kb promoter regions of the differentially up-regulated genes in the *ugmA* mutant using an in house developed transcription factor binding site finder program, indicated overrepresentation of genes with RlmA or SteA binding sites. The importance of the RlmA and SteA transcription to induce cell wall remodelling genes is currently under investigation by constructing a *rlmA-ugmA* and *steA-ugmA* double mutants.

106. The use of the parasexual cycle and bulk segregant analysis followed by high-throughput sequencing to characterize a sclerotia-forming mutant in *Aspergillus niger*. Arthur Ram, Anne-Marie Burggraaf-van Welzen, Jing Niu, Thomas Jørgensen, Mark Arentshorst. Molecular Microbiology and Biotechnology, Leiden University, Institute Biology Leiden, Leiden, Netherlands.

Much of our current understanding in fungal growth and development is derived from forward genetic screens to select developmental mutants. Identification of the molecular basis for a particular phenotype in UV-generated mutants is tedious and time consuming. Whole genome sequencing (WGS) provides an effective alternative to identify the molecular lesion in a mutant isolated from a genetic screen. We have successfully used the so called "bulk segregants analysis" approach in combination with WGS to characterize the molecular basis for a hypersclerotial mutant (*scl-2*) in *A. niger*. Since *A. niger* has no sexual cycle, we used the parasexual cycle to obtain segregants. In the bulk-segregant analysis approach, the mutant of interest is crossed to a wild-type strain and segregants displaying the phenotype of interest are pooled and DNA from this pool of segregants is sequenced using a deep sequencing technology (e.g. Illumina). In addition, the genomes of the parental strains were also sequenced and SNPs and indels were identified. SNPs and indels between the parental strains not related with the phenotype, have a 50% chance to be present in the pool; SNPs and indels responsible for the phenotype or closely linked to the mutation responsible for the phenotype, are conserved in the pool. Sclerotia formation was found to be caused by a non-sense mutation in the Zn(II)2Cys6 domain of an until now unknown transcription factor. Subsequent complementation analysis and targeted deletion are in progress to confirm its role as a potential repressor of sclerotia formation in *A. niger*. The opportunity to use WGS approaches to pinpoint the molecular lesion in a mutated strain isolated from a genetic screen will speed up genetic identification making is suitable for new approaches of near complete mutational saturation of a biological process and the resulting unravelling of entire genetic pathways and networks.

107. Micro-colony heterogeneity in liquid cultures. Brand Recter, Wieke Teertstra, Han Wösten. Department of Biology, Utrecht University, Utrecht, The Netherlands.

Aspergillus niger is an important cell factory for enzymes and organic acids. Fungal morphology is a key contributor for productivity. The fungus is grown in large scale bioreactors in industry. Morphology can range from dispersed mycelium to mm-scale micro-colonies. Here we studied the morphology of *A. niger* mycelium in liquid shaken cultures using a COPAS-plus device, equipped with a 1 mm nozzle. COPAS-analysis showed that micro-colonies are not uniformly distributed when conidia that were harvested 1 day after their formation were used for inoculation. Statistical analysis showed a population of large and small micro-colonies. In contrast, inoculation with spores that had been harvested 7 days after their formation resulted in a normal size distribution within the culture. Initial spore concentration also impacted heterogeneity of the mycelium in a liquid shaken culture. When inoculated with a 7-fold higher spore concentration, smaller micro-colonies were formed with a normal size distribution. In the next set of experiments we assessed the mechanisms of micro-colony formation. Using GFP and dTomato labelled spores we showed that, if both types are mixed before spore germination, all micro-colonies have a mixture of both types of fluorescence. However, when these strains were mixed after germination, only 15% of the micro-colonies showed both types of fluorescence. This shows that secondary aggregation is less frequent when compared to conidial aggregation.

108. Characterization of septum association of a Woronin body-tethering protein Leashin in *Aspergillus oryzae*. Pei Han, Jun-ichi Maruyama, Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

Woronin body, a Pezizomycotina-specific organelle, is tethered to the septum in normal growth condition, and it plugs the septal pore in response to cellular wounding, preventing the excessive loss of cytoplasm. Recently, we identified a Woronin body tethering protein AoLAH in *Aspergillus oryzae*[1)]. AoLAH is a single polypeptide with 5,727 amino acids; it is composed of conserved N- and C-terminal regions and a long non-conserved middle region. We found that AoLAH N-terminal and C-terminal regions function for Woronin body association and tethering to the septum, respectively. AoLAH middle region confers positional flexibility to the tethered Woronin bodies. However, how the AoLAH C-terminal region associates specifically with the septum is not known. The aim of this study is to investigate the mechanism for septal association of AoLAH.

AoLAH C-terminal region consists of 1,018 amino acids. According to secondary structure analysis, the former 505 amino acids of this region is mostly structurally disordered while in the latter 513 amino acids several secondary structures such as α-helix and β-sheet were predicted. This structure prediction is similar to those of the C-terminal region of LAH proteins from other *Aspergillus* species. AoLAH C-terminal region fused with EGFP was closely associated with the septum as well as hyphal tip, and it was also observed as moving tubular structures formed in a microtubule-dependent manner. This gives the possibility that AoLAH C-terminal region could be functionally related with microtubules. After deleting the former 505 amino acids with predicted disordered feature, no motile tubular structures were observed, indicating that this part is needed for tubular structure formation and movement along hyphae. However, the localization associated at the septum and hyphal tip was still detected. Thus, the latter 513 amino acids with α-helix and β-sheet structures are sufficient

to function for association to the septum and hyphal tip. This suggests the AoLAH C-terminal region may target to the septum and hypahl tip in a similar mechanism. 1) Han *et al*. (2014) *Eukaryot. Cell* 13: 866-77.

109. Investigation of molecular mechanism regulating light-dependent repression of conidiation in *Aspergillus oryzae*. Jun-ichi Maruyama, Helge M. Dietrich, Feng Jie Jin, Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

The domesticated filamentous fungus *Aspergillus oryzae* is an important for Japanese fermentation industry. Its conidia are of high interest for industrial use such as *tane-koji* (*koji* starter) producing companies and fermentation companies. Although other *Aspergillus* species such as *Aspergillus nidulans* predominantly undergo conidiation under light illumination[1)], *A. oryzae* forms conidia in dark, but conidiation efficiency is reduced in light[2)]. It could be speculated that *A. oryzae* strains forming conidia in dark have been selected due to the industrial necessity to grow them in dark room. How the fungus reacts to light in this reverse way has not yet been understood. To investigate this question, we attempted to molecularly uncover the light response mechanism in *A. oryzae*.

When LED illumination was used, blue light was sufficient for the repression of conidiation, whereas red light had no impact. Subsequently, deletion strains of the genes for putative blue light (*AolreA*) and red light (*AofphA*) receptors were generated. White and blue light illuminations resulted in the repression of conidiation for the wild-type and Δ*AofphA* strains. Deletion of *AolreA* gene caused a complete attenuation of the light-induced repression. These results indicate that AoLreA but not AoFphA is essential for light-dependent repression of conidiation. RT-PCR analysis was performed for *brlA* gene encoding a positive conidiation regulator. White and blue light illumination decreased the *brlA* mRNA amount in the wild-type and Δ*AofphA* strains, which is consistent with their repressive conidiation phenotypes in light. In contrast, no light-dependent decrease of *brlA* mRNA amount was observed in the absence of AoLreA. These data indicate that AoLreA governs the light-induced repression of conidiation in *A. oryzae*, which is an opposite way to that of *A. nidulans* LreA.

1) Ruger-Herreros *et al*. (2011) *Genetics* 2) Hatakeyama *et al.* (2007) *Biosci. Biotechnol. Biochem.*.

110. Functional characterization of two proteins potentially involved in response to fludioxonil in *Botrytis cinerea*: new virulence factors? Jaafar Kilani[1,2], Colette Audeon[1], Sabine Fillinger[1]. 1) INRA, AgroParis Tech, UMR1290, BIOGER, BP01, F78850 Thiverval-Grignon, France; 2) Université Paris Sud, 91400 Orsay, France.

Fungi adapt rapidly to their environment due to signalling pathways like those involving mitogen activated protein kinase (MAPK). In the necrotrophic pathogenic fungus *Botrytis cinerea*, the fungicide fludioxonil actives Sak1 and Bmp3 MAPKs which are involved in osmoregulation, cell wall integrity, development and pathogenicity. In order to understand the perception and transduction of the fludioxonil signal, two proteins have been studied, Pom1 and PhnA which were identified from a comparative phosphoproteomic analysis using fludioxonil.

Pom1 is a protein kinase, which was characterised in *Schizosaccharomyces pombe* and which is involved in the synchronization between growth and cell division. The function of this protein has not been studied in filamentous fungi so far. Thus in *B. cinerea*, the *pom1* deletion mutant showed reduced growth and an early stop of necrosis development *in planta*. It appears that *pom1* has a crucial role during the infectious process but not during plant penetration. Moreover preliminary microscopic studies have shown a likely increase of septation and branching indicating a potential role of Pom1 in the polar growth in *B. cinerea*.

In eukaryotes, the phosducin-like protein PhnA allows the dimerization between the subunits Gβ and Gγ in the G-protein signalling pathway. The *phnA* deletion mutant did not show necrosis *in planta*, but reduced *in vitro* growth and absence of conidia and sclerotia production. Thus the deletion of *phnA* in *B. cinerea* showed that this gene is involved in vegetative growth, pathogenicity, and in development.

The functional analysis of the two genes *pom1* and *phnA* highlighted two new pathogenicity factors. Their involvement in fludioxonil signal transduction remains to shown.

111. The NADPH oxidase complex in *Botrytis cinerea* - New functions, members and the potential link to essential ER functions. Robert Marschall, Ulrike Siegmund, Paul Tudzynski. Institute of Plant Biology, University of Münster, Münster, Germany.

Reactive oxygen species (ROS) are produced in conserved cellular processes either as byproducts of the cellular respiration in mitochondria or as a support for defense mechanisms, signaling cascades or cell homeostasis. Their most common enzymatic producers are NADPH oxidases (Nox). In fungi several subunits of these complexes have been identified and were shown to be involved in sexual differentiation, pathogenicity and therewith in plant-fungi interactions.

Botrytis cinerea, also known as the gray mold fungus, is a necrotrophic plant pathogen with a broad host spectrum containing economically important crops like tomatoes and grapes. In this phytopathogenic fungus two NADPH oxidase isoforms (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified (Segmueller et al., 2008). *For B. cinerea* recently a new component was identified. The putative ER protein BcNoxD interacts directly with BcNoxA, and therewith presents for the first time a direct interaction between the catalytic subunits of the Nox-complex and other cellular components in fungi.

Deletion mutants of BcNoxA and BcNoxD display an identical phenotype regarding pathogenicity, sclerotia and conidia formation. Interestingly, both proteins seems to be not essential for the production of superoxide, since it was shown by the use of a new ROS detection agent, that the knock out has no effect on external ROS levels and superoxide production during the penetration of onion epidermal layers. Complementation studies revealed functions of the catalytic subunit BcNoxA inside the ER. Putative effects on the redox state can be displayed by the redox sensitive biosensor RoGFP2 as an efficient tool for the measurement of redox state changes (Heller et al., 2012).

Heller J, Meyer AJ, Tudzynski P (2012) Mol Plant Pathol. 13(8):935-47

Segmueller N. et al., (2008) Mol Plant Microbe Interact 21: 808-808-819.

112. Two key enzymes, one product – DHN melanin biosynthesis in the plant pathogen *Botrytis cinerea* is due to two differentially expressed PKS-encoding genes. Julia Schumacher. IBBP, WWU Münster, Schlossplatz 8, 48143 Münster, Germany.

The biosynthesis of the dark pigment melanin involves the polymerization of DHN (1,8-dihydroxynaphthalene) that is produced via THN (1,3,6,8-tetrahydroxynaphthalene). Multiple routes for the assembly of THN by non-reducing polyketide synthases (PKSs) have evolved that are due to different properties of the C-terminal thioesterase domains to release hepta- or hexaketides. PKS from *Colletotrichum lagenarium* forms THN directly, while *Aspergillus fumigatus* ALB1 and *Wangiella dermatitidis* PKS1 form hexaketidic intermediates that are deacylated by AYG1 to THN. The subsequent conversion of T4HN to DHN comprises two rounds of enzymatic reduction and one dehydration step, and is carried out in a similar fashion in all fungi. *Botrytis cinerea*, the causal agent of gray mold disease and a member of the Leotiomycetes, incorporates DHN melanin in the conidia and the sclerotia. In contrast to other fungi, *B. cinerea* possesses two highly similar PKS-encoding genes (*bcpks12*, *bcpks13*), while single copies for the other melanogenic genes exist. The relevance of having two copies of the key enzyme appears obscure when both enzymes are functional. However, considering the differential expression pattern of *bcpks12* (during sclerotial development) and *bcpks13* (during conidiation), we hypothesized that BcPKS12 and BcPKS13 are responsible for sclerotia- and conidia-specific melanin biosynthesis, respectively. Indeed, PKS deletion mutants are affected in sclerotial (Δ*bcpks12*) or conidial (Δ*bcpks13*) melanization while mutants of the core pathway (conversion of T4HN) are impaired in total melanization leading to the accumulation of orange shunt products in both conidia and sclerotia. Phylogenetic analyses indicate that BcPKS13 groups together with WdPKS1 while BcPKS12 groups with PKSs of melanin-producing Sordariomycetes suggesting that BcPKS13 might be the native key enzyme and that BcPKS12 has been achieved via horizontal gene transfer. This hypothesis is corroborated by the fact that the gene next to *bcpks12* encoding the "Sclerotial Melanin Regulator" (BcSMR1) is more closely related with orthologs from the Sordariomycetes (e.g. *Magnaporthe oryzae* PIG1) than with those from the Dothideomycetes (e.g. *Alternaria alternata* CMR1).

113. Study of clathrin in the phytopathogenic fungus *Botrytis cinerea*. Eytham SOUIBGUI[1,2], Nathalie POUSSEREAU[1], Marie-Pascale LATORSE[2]. 1) University of Lyon, UMR5240, France; 2) BAYER SAS, 14 impasse Pierre Baizet, 69263 Lyon, France.

Endocytosis is the mechanism involved in internalization of molecules or particles into the cell. This mechanism is mainly required for nutriments uptake, receptors mediated endocytosis, regulation of signaling molecules, plasma membrane turnover and cell polarity. Different types of endocytosis pathways have been described and the most characterized pathway is the clathrin-mediated endocytosis. This mechanism is widely described in mammalians, conserved from yeast to humans and it is known to be essential in higher organisms.

The formation of clathrin-coated vesicles results from five stages corresponding to initiation, cargo selection, coat assembly, vesicle scission and uncoating. They imply more than 60 proteins recruited in an ordered sequence. The main component of the coat is clathrin which forms a polymeric mechanical scaffold on the vesicle surface.

Clathrin function is not limited to endocytosis since it is also crucial in many other cellular processes such as protein secretion from the trans-Golgi network, ESCRT-dependent cargo sorting at endosomes and mitosis (McMahon & Boucrot, 2011).

Few studies have been performed on clathrin in filamentous fungi but exploration of the fungal genomes highlights the conservation of clathrin mediated endocytosis genes. A focus on expression data and localization of clathrin in *B.cinerea* will be presented.

McMahon & Boucrot, 2011, nature reviews 12: 517-533.

114. Stress signaling in *Botrytis cinerea:* The response regulator BcSkn7. Anne Viefhues, Ines Schlathoelter, Paul Tudzynski. IBBP, University of Muenster, Muenster, Germany.

In the course of plant infection pathogens trigger an oxidative burst, which is part of the plants early defense reaction. The necrotrophic plant pathogen *Botrytis cinerea* is known to contribute actively to the release of reactive oxygen species (ROS). ROS have an ambivalent role as they are on the one hand toxic and responsible for damages of biological molecules and on the other hand they are important second messengers. For the integration and transmission of these signals several stress responsive components are necessary to evoke the appropriate response.

Two key players in the oxidative stress response are the transcription factor Bap1[1] and the response regulator BcSkn7. Phenotypic analysis of *bcskn7* and *bap1bcskn7* deletion mutants showed alterations in differentiation, virulence and gene expression. Δbcskn7 is reduced in vegetative growth and affected in the formation of reproduction structures. The mutant is highly sensitive to oxidative stress, as well as reacts to temperature and osmotic changes. Modifications in the composition of the cell wall could be detected, indicated by changed gene expression, reduced protoplast formation and sensitivity to cell wall or membrane stressors. Furthermore, an enhanced secretion of ROS could be noticed. Virulence of the mutants based on conidia is not affected, however mycelium derived infections are defective. This effect is even more severe for ΔΔbap1bcskn7 and is probably due to a reduced penetration ability of the infection cushions. Expression analyses revealed a strong influence of Bap1 and BcSkn7 on the regulation of oxidative stress responsive genes. In a Y1H approach a direct binding to the promotors of *gsh1* and *grx1* by Bap1 and of *glr1* by BcSkn7 could be verified. Next steps include the examination of a direct interaction between Bap1 and BcSkn7 as they seem to act in concert in gene regulation.

[1]Temme N. and Tudzynski P. (2009) Does *Botrytis cinerea* Ignore H_2O_2-Induced Oxidative Stress During Infection? Characterization of *Botrytis* Activator Protein 1. *MPMI* **22 (8)**: 987–998.

115. Depletion of the mitotic kinase Cdc5p in *Candida albicans* results in the formation of elongated buds that switch to the hyphal fate over time in a Ume6p-dependent manner. Amandeep Glory[1], Chloë Triplet van Oostende[2], Anja Geitmann[2], Catherine Bachewich[1]. 1) Biology Department, Concordia University, Montreal, Quebec, Canada; 2) Institut de recherche en biologie végétale, Université de Montréal, Montreal QC, Canada.

The fungal pathogen *Candida albicans* differentiates between yeast, hyphae and pseudohyphae in order to enhance survival in the human host. Environmental cues induce hyphal development and expression of hyphal-specific genes. *C. albicans* can also form filaments in response to yeast cell cycle arrest, in the absence of environmental cues, but the nature of these cells and their mechanisms of formation are less clear. We previously demonstrated that depletion of the mitotic polo-like kinase Cdc5p resulted in the production of filaments under yeast growth conditions that were distinct from hyphae with respect to several criteria, yet maintained polarized growth and expressed hyphal-specific genes at later stages of development. In order to clarify the nature of these growth forms and their relationship to true hyphae, we conducted time course-based investigations of aspects of the polar growth machinery, which can help distinguish cell types. In Cdc5p-depleted cells, the Cdc42p GAP Rga2p became hyper-phosphorylated, as in true hyphae, but this was observed at only later stages of *CDC5* repression. Further, the hyphal-specific genes *HWP1, UME6* and *HGC1* were strongly induced at approximately the same time as Rga2p phosphorylation. The tips of some later-stage filaments also demonstrated the hyphal-specific Spitzenkörper-like localization of the myosin light chain Mlc1p. *HWP1* expression was dependent on Ume6p, and absence of Ume6p or Hgc1p influenced late-stage filament diameter and integrity. Finally, polarized growth and *UME6* expression in Cdc5p-depleted cells were independent of the transcription factor Hms1p. Thus, depleting Cdc5p may generate elongated yeast buds that switch to the hyphal fate over time through a mechanism that involves *UME6* induction, possibly in response to maintenance of polarized growth. The results expand on the multiple strategies with which *C. albicans* can modulate growth mode and expression of virulence determinants.

116. Heterokaryon analysis of a Cdc48-like gene, *CpCdc48*, from the chestnut blight fungus *Cryphonectria parasitica* demonstrates it is essential for cell division and growth. Y.-H. Ko[1], J.-M. Kim[2], Y.-S. Jang[1], D.-H. Kim[1]. 1) Institute for Molocular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju-si, Jeollabuk-do, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeollabuk-do, South Korea.

Functional analysis of a cell division cycle 48 (CDC48) ortholog, *CpCdc48*, from *Cryphonectria parasitica* was attempted via construction of a *CpCdc48*-null mutant. Genotype analysis revealed that the putative *CpCdc48*-null mutant was a heterokaryotic transformant containing two different types of nuclei (i.e., one with the wild-type *CpCdc48* allele and the other with the *CpCdc48*-null mutant allele). Although stable mycelial growth of the heterokaryotic transformant was observed on media containing hygromycin B, neither germination nor growth of the resulting spores was observed on the selection media suggesting that the *CpCdc48* gene is essential for growth. Microscopic analysis of germinated conidia from the heterokaryon demonstrated that, although there were normal germinating spores due to the wild-type conidia, there were many residual conidia that did not germinate. However, with prolonged incubation, non-germinating conidia began to swell into gigantic globose spores. DAPI staining of these gigantic spores revealed the presence of multiple nuclei without further germination. These gigantic conidia were not stable and underwent autolysis with further incubation. These findings indicate that the cloned *CpCdc4*8 gene is responsible for delayed cell cycle during spore germination resulting in some karyokinesis but not following spore cytokinesis. Thus, *CpCdc48* is essential for cell division and growth.

117. An S-phase checkpoint is necessary for appressorium-mediated plant infection in the rice blast fungus *Magnaporthe oryzae*. Miriam Oses-Ruiz, Wasin Sakulkoo, Nicholas J. Talbot. University of Exeter, Exeer, Devon, United Kingdom.

The rice blast fungus, *Magnaporthe oryzae*, elaborates a specialized dome-shaped infection structure called an appressorium. Initiation of appressorium development requires a switch from apical to isotropic growth, which is regulated by a G1 to S phase cell cycle transition. Appressorium maturation is coupled to a single round of mitosis in the germ tube and generation of enormous turgor. This leads to septin-dependent re-orientation of the F-actin cytoskeleton, which leads to penetration peg formation and plant cell invasion. Here, we present evidence suggesting that appressorium-mediated plant penetration is regulated by an S-phase checkpoint that operates in the appressorium. Progression of the appressorial nucleus into G2 is necessary for cytoskeletal re-polarisation at the base of the appressorium and for penetration peg formation and plant infection. Moreover, we also show that DNA checkpoint kinases are necessary for the S-phase mediated processes involved in initial appressorium development, but that they are dispensable for the penetration S-phase checkpoint. The S-phase checkpoint required for plant penetration operates independently of the conventional DNA damage and repair response. Generation of an analogue-sensitive mutant allele of Cdc28 also provided new insight into the mechanism of cell cycle regulation of appressorium-mediated plant penetration in the rice blast fungus. An integrated model for the operation of cell cycle regulation and the

control of appressorium morphogenesis and appressorium-mediated plant penetration will be presented.

118. ChIP-seq Analysis Identifies Target Genes of Transcription Factor PRO1 that Regulates Multicellular Development in *Sordaria macrospora*. Eva Steffens, Ines Teichert, Ulrich Kück. General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany.

Sordaria macrospora is a homothallic ascomycete, that has been studied as a model organism for fungal sexual development since the late 50s. In forward genetic approaches several mutants were generated showing sterile phenotypes. In this collection of mutants we recently characterized mutant pro1, which generates only immature (protoperithecia) but never mature fruiting bodies (perithecia). The mutant carries a gene deletion of the transcriptional regulator PRO1, which is characterized by a $Zn(II)_2Cys_6$ binuclear cluster, a DNA binding motif at the N-terminus, and a putative nuclear localization signal (NLS). Thus it is related to the well-characterized transcription factor GAL4 from yeast. Using a GFP-tagged version of PRO1, we were able to demonstrate a nuclear localization. The construct was also used for chromatin immunoprecipitation coupled with high throughout sequencing (ChIP-seq) to identify the genome-wide distribution of transcription factor binding sites. We were able to identify different MAP kinases and a scaffold protein of the cell wall integrity pathway as putative PRO1 target genes. It was already shown that several of the target genes are involved in sexual differentiation of *Sordaria macrospora*. A PRO1 DNA-binding motif was identified in promoter regions of some of these target genes and verified by EMSA analysis. We also aim to identify interaction partners of PRO1 using immunoprecipitation together with mass spectrometry. The results of both techniques will improve our understanding of a regulatory network controlling cellular differentiation.

119. Interactions of the *Candida albicans* chitin synthases at septation sites. Amy Ross, Maria Spyrou, Megan Lenardon. Aberdeen Fungal Group, University of Aberdeen, Aberdeen, United Kingdom.

As fungal cells divide, they form a septum which acts as a stabilising barrier between the mother and daughter cells in yeast, and between compartments in hyphae. In most fungi, the primary septum is made of chitin, the synthesis of which is essential for the viability of the fungus. Most fungi have several enzymes which make chitin. *Candida albicans* has four chitin synthase enzymes (Chs1, Chs2, Chs3 and Chs8), all of which are present at sites of septation before cytokinesis. The currently accepted view is that the class IV enzyme (Chs3) synthesises a chitin ring at septation sites onto which the essential class II enzyme (Chs1) synthesises the primary septal plate as the actomyosin ring contracts between the two split septin rings. The role of the class I enzymes (Chs2 and Chs8) at septation sites is unknown.

We are probing the interactions of the *C. albicans* chitin synthases at septation sites in using live-cell fluorescence imaging and membrane yeast two-hybrid assays. Single strains expressing multiple fluorescently-tagged chitin synthases are being used to determine the temporal and spatial recruitment of the chitin synthases to septation sites, and co-localisation of proteins is being used as an indicator of potential protein-protein interactions. Physical interaction between these protein pairs is then being tested in a split-ubiquitin membrane yeast two-hybrid assay system. The information gathered in these experiments will be used to build a model of the *C. albicans* "septasome" and to answer fundamental questions about how chitin synthesis is achieved at septation sites.

120. *CoWHI2*, the homolog of stress response regulator *WHI2* of *Saccharomyces cerevisiae*, is involved in induction of host defense response and regulation of hemibiotrophic infection in *Colletotrichum orbiculare*. H Ken, Y Kubo. kyoto prefecural university, laboratory of plant pathology, graduate school of life and enviromental science, kyoto, Japan.

Colletotrichum orbiculare small GTP-binding protein CoRas2 positively regulates activation of cAMP-PKA and MAPK CoMekk1-Cmk1 signaling pathway involved in infection-related morphogenesis, and shows dynamic cellular localization in the process of infection. *In Saccharomyces cerevisiae*, stress response regulator *WHI2* contributes to stabilization of F-actin structure in response to nutritional sensing, and F-actin dynamics require for proper localization of Ras protein. Therefore, to elucidate relationship of spatiotemporal regulation of CoRas2 and infection-related morphogenesis, we functionally analyzed *C. orbiculare CoWHI2*, the homolog of *WHI2* of *S. cerevisiae*. First, we observed the pathogenesis and morphogenesis of the *cowhi2* mutant. The *cowhi2* mutant showed attenuate pathogenicity on the cucumber cotyledons, and formed necrotrophic like narrow penetration hyphae at the primary infection stage unlike biotrophic penetration hyphae in the wild-type, suggesting that *CoWHI2* may be involved in transition from biotrophic stage to necrotrophic stage during infection. Next, to elucidate whether host plant defense response is affected by the change of infection mode in the *cowhi2* mutant, we observed callose deposition and ROS accumulation beneath appressoria of the *cowhi2* mutant. As expected, the *cowhi2* appressoria were accompanied by stronger callose deposition and ROS accumulation compared with the wild-type. This data indicated that *cowhi2* mutation is involved in rapid and increased induction of host defense response. Finally, to check whether the *cowhi2* mutant accelerates transition from biotrophic stage to necrotrophic stage, we evaluated expression of *PR* gene *Cucumis sativus DEFENSIN19* in the initial phase of epidermal cell infection. The expression of cucumber *DEFENSIN19* inoculated with the *cowhi2* mutant was significantly upregulated compared with the wild-type, suggesting that *CoWHI2* could be a key factor for hemibiotrophic infection.

121. *Colletotrichum orbiculare* MOR signaling pathway is involved in appressorium development triggered by cutin monomers from the host plant exudate. S. Kodama[1], A. Sakaguchi[1], I. Miyashita[1], T. Ishii[2], H. Miyoshi[3], Y. Kubo[1]. 1) Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan; 2) Laboratory of Pomology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan; 3) Laboratory of Biofunction Chemistry, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

In many plant pathogenic fungi, morphogenesis of infection structures is triggered by physical or chemical signals from plant surface. We previously reported that cucumber anthracnose fungus *Colletotrichum orbiculare CoKEL2*, a *Schizosaccharomyces pombe tea1* homologue is essential for proper morphogenesis of appressoria on artificial substrate but is dispensable for appressorium formation on host plant surface. Our results suggested that there could be a bypass pathway that transduces plant-specific signals for appressorium formation

independent of *CoKEL2*. To determine specific components of the plant-specific signaling pathway, we performed the screening of insertional mutants in *cokel2Δ* background and identified *CoPAG1*, a *Saccharomyces cerevisiae PAG1* homologue as a mutated gene of a mutant. Pag1 is one of the components of MOR [morphogenesis-related NDR (nuclear Dbf2-related) kinase network], a signaling cascade that is involved in the maintenance of cell polarity and morphogenesis. Targeted gene deletion analysis revealed that CoPag1 is a key component of plant-specific signaling pathway. In addition, cellular localization of CoPag1 responds to biological signals during appressorium development. In *S. cerevisiae* MOR, Pag1 facilitate activation of the NDR kinase Cbk1. Constitutive activation of CoCbk1 led to suppression of *copag1Δ* phenotype, suggesting that CoPag1 functions *via* MOR pathway in *C. orbiculare.* A cutin monomer isolated from cucumber exudate restored normal appressorium formation of *cokel2Δ* on artificial substrate, whereas *copag1Δcokel2Δ* formed abnormal appressoria in the presence of the cutin monomer. Our data demonstrate that MOR pathway plays a crucial role in appressorium formation triggered by the cutin monomer from host plant surface.

122. The HLH transcription factor *ndrA*, which is necessary for conidiation, negatively controls sclerotia formation in *Aspergillus flavus*. Kap-Hoon Han[1], Mohammed A. Abdo Elgabbar[1], Dong-Min Han[2]. 1) Pharmaceutical Engineering, Woosuk University, Wanju, Jeonbuk, 565-701, South Korea; 2) Division of Life Science, Wonkwang University, Iksan, 570-749, South Korea.

Aspergillus flavus is a saprophytic and pathogenic fungus that can infect animals and humans directly or indirectly by its secondary metabolites. It mainly reproduces clonally by means of conidia (asexual spores), although sexual developmental process has been recently reported. In eukaryotes, the helix-loop-helix (HLH) transcriptional factors play an important role in the developmental processes. One of these factors is *Aspergillus nidulans* HLH transcription factor *ndrA*, which is involved in the early stage of conidiophore development and sexual development. Previous unpublished results showed that expression of the *ndrA* (NsdD-Dependent Regulator) gene is largely affected by a GATA factor NsdD, and is negative regulator of sexual development as well as positive regulator of asexual development. By BLASTP of *A. niduans* NdrA, we identified the orthologue of *ndrA* (*AflndrA*) in *A. flavus*. The deletion of *AflndrA* resulted in almost absence of conidia yet abundant production of sclerotia. The complementation of *AflndrA* deleted strain by the intact *AflndrA* ORF has restored the conidiation as in the wild type with diminishing sclerotia. Moreover, we found that, *AflndrA* dose not affect the aflatoxin production as well as the antifungal drug sensitivity or resistance. The expression of *AflndrA* is upregulated at 12 hours under asexual development favorable condition. Taken together, the *AflndrA* gene could be considered as a one of the conidiation-critical and sclerotia controlling genes in *A. flavus*..

123. *Aspergillus fumigatus* HLH transcription factor, *AfundrA*, is required for conidiation. Mohammed A. Abdo Elgabbar[1], Sang-Cheol Jun[2], Jong-Hwa Kim[1], Dong-Min Han[3], Kap-Hoon Han[1]. 1) Pharmaceutical Engineering, Woosuk University, Wanju, Jeonbuk, 565-70, South Korea; 2) Division of Biological Sciences, Chonbuk National University, Jeonju, 561-756, South Korea; 3) Division of Life Science, Wonkwang University, Iksan, 570-749, South Korea.

Asexual reproduction and conidiation in the Aspergillus spp. is a mean by which progeny arise from a single parent, and inherit the parental genes only. In an opportunistic pathogenic fungus *Aspergillus fumigatus*, conidia are the primary causative agent of invasive aspergillosis. The helix-loop-helix (HLH) transcriptional factors that control cell growth and differentiation are considered as key regulators for a wide range developmental processes. In a model fungus *Aspergillus nidulans*, one HLH gene, named *ndrA*, which is regulated by NsdD GATA factor, has been isolated and characterized as a negative regulator of sexual development as well as a positive regulator of asexual development. To study conserved and divergent role of the *A. nidulans ndrA*, we performed BLAST search and identified the *A. fumigatus* ortholog *AfundrA* gene, which is its knockout made this fungus unable to produce the conidia. The *AfundrA* complemented strain was able to produce numerous amounts of conidia, which is as same as the wild type strain. Northern analysis showed that the *AfundrA* gene was highly expressed in the early stage in the conidiation. There was no difference between the wild type and *AfundrA* deletion mutant when they subjected to antifungal sensitivity test. Moreover, there were no big differences in the growth rates between the wild type and *AfundrA* deletion mutant. Taken together, in *A. fumigatus AfundrA* gene plays a pivotal role in controlling conidiation.

124. Genetic dissection of cell-cell fusion and nuclear fusion during bisexual and unisexual reproduction of *Cryptococcus neoformans*. Ci Fu, Soo Chan Lee, Joseph Heitman. Molecular Genetics and Microbiology, Duke University, Durham, NC.

The opportunistic human fungal pathogen *Cryptococcus neoformans* has two modes of sexual reproduction: canonical bisexual reproduction between partners of opposite mating-types, **a** and alpha, and unisexual reproduction between partners of only one mating-type. Unisexual reproduction can function similar to bisexual reproduction by admixing parental genetic material to generate genetic diversity among offspring, which may contribute to explain the evolutionary significance of the unisexual reproduction mode in global *Cryptococcus* populations that are predominantly alpha mating-type. During the bisexual reproduction cycle, mating partners undergo cell-cell fusion, produce a hyphal dikaryon, and then undergo nuclear fusion in the basidium to form a diploid precursor to meiosis. However, the mechanisms of the haploid-diploid ploidy shift during the unisexual reproduction cycle are less well understood and may involve endoreplication or nuclear fusion. Previous studies have shown that cell-cell fusion can occur between mating partners of the same mating-type at low frequency, and that deletion of the nuclear fusion gene *KAR7* impairs unisexual reproduction. In this study, we identified the *C. neoformans* orthologs of the *PRM1* and *KAR5* genes that govern cell-cell fusion and nuclear fusion respectively during mating of the model budding yeast *Saccharomyces cerevisiae*. To understand how cell-cell fusion and nuclear fusion contribute to the ploidy duplication event during the bisexual and unisexual reproduction cycles, we are characterizing the *prm1* and *kar5* deletion mutants of *C. neoformans* and identifying their functions during both unisexual and bisexual reproduction. Our preliminary findings, taken with previous studies, provide evidence that cell-cell fusion and nuclear fusion are both essential for bisexual reproduction and both also contribute significantly to unisexual reproduction.

125. Impact of protein palmitoylation on the virulence potential of *Cryptococcus neoformans*. Connie Nichols, Kyla Selvig, Andrew Alspaugh. Duke University Medical Center, Durham, NC, USA.

In the opportunistic human fungal pathogen *Cryptococcus neoformans* a Ras1 signaling cascade mediates cell morphology and cytokinesis in response to mild stress, such as growth as 37°C. Localization of Ras1 to the plasma membrane is required for this signaling pathway. Previously we found that the palmitoylation of Ras1 specifies localization to the plasma membrane. Palmitoylation is the post-translational addition of a palmitoyl group to cysteine and is catalyzed by a conserved family of protein S-acyltransferase (PAT) enzymes. To identify the Ras1 PAT in *C. neoformans* we have identified and characterized seven putative PATs. Although there is some degree of functional redundancy in this protein family, we have identified Cpt4 (***C****ryptococcus* **p**almitoyl **a**cyltransferase 4) as a Ras1-specific PAT. Deletion of the *CPT4* gene results in reduced Ras1 protein palmitoylation, altered Ras1 localization, impaired growth at 37°C, and reduced virulence.

126. Ssn3, a cyclin-dependent protein kinase, modulates unisexual reproduction in *Cryptococcus neoformans*. Yu-Lin Shi, Kuang-Hung Liu, Wei-Chiang Shen. Department of Plant Pathology and Microbiology, National Taiwan University , Taipei, Taiwan.

Cryptococcus neoformans is an environmental and pathogenic basidiomycete containing two different mating types, *MAT***a** and *MAT*α. Under nitrogen starvation conditions, two different sexual reproduction events, bisexual reproduction and unisexual reproduction, are observed in *C. neoformans*. In the processes of bisexual reproduction, *MAT***a** and *MAT*α cells undergo cell fusion and generate dikaryotic filaments. Subsequently, basidia and basidiospores are formed at the tips of dikaryotic filaments to complete the sexual cycle. Interestingly, similar sexual differentiation, so called unisexual reproduction, can occur in only the *MAT*α cells without *MAT***a** cells involved. This unique event has been implicated to contribute to the generation of recombinant progeny responsible for the recent outbreak of disease. *C. neoformans* Ssn8, a putative cyclin-like component of the RNA polymerase II holoenzyme, was identified in a genetic screen and shown to play negative roles in diverse physiological processes including unisexual reproduction. To further dissect the molecular networks of same-sex mating in *C. neoformans*, we here characterized a putative interacting component of Ssn8, the cyclin-dependent protein kinase Ssn3. Mutation of the *SSN3* gene reproduced the sexual reproduction and other phenotypes of *ssn8* mutants, and hyperfilamentation during unisexual reproduction was observed in the *MAT*α and *MAT***a** *ssn3* mutant strains. Yeast two-hybrid assay confirmed that Ssn8 physically interacted with Ssn3. These results suggest unisexual reproduction is regulated by Ssn8/Ssn3 complex in *C. neoformans*. Although filamentation the phenotypes of *ssn3* and *ssn8* mutants were similar, delayed and reduced sporulation in bilateral *ssn3* mutant cross were observed. To investigate how *SSN8/SSN3* regulates same-sex mating process, we conducted genes expression studies in *ssn3*/*ssn8* or other mutants background. Possible the regulatory networks of same-sex mating will be discussed in *C. neoformans*.

127. Unisexual reproduction drives meiotic recombination and phenotypic and karyotypic plasticity in *Cryptococcus neoformans*. Sheng Sun[1], R. Blake Billmyre[1], Piotr A. Mieczkowski[2], Paul Magwene[3], Joseph Heitman[1]. 1) Molecular Genetics & Microbiology, Duke University Medical Center, USA; 2) Department of Biology, University of North Carolina, Chapel Hill, USA; 3) Department of Biology, Duke University, USA.

In fungi, unisexual reproduction, where sexual development is initiated without the presence of two compatible mating type alleles, has been observed in several species that can also undergo traditional bisexual reproduction, including the important human fungal pathogens *Cryptococcus neoformans* and *Candida albicans*. While unisexual reproduction has been well characterized qualitatively, detailed quantification is still lacking. Here, we analyzed meiotic recombination during α-α unisexual and **a**-α bisexual reproduction of *C. neoformans*. We found that meiotic recombination operates in a similar fashion during both modes of sexual reproduction. Specifically, we observed that in α-α unisexual reproduction, the numbers of crossovers along the chromosomes during meiosis, recombination frequencies at specific chromosomal regions, as well as meiotic recombination hot and cold spots, are all similar to those observed during **a**-α bisexual reproduction. Additionally, we found diploid meiotic progeny were produced at similar frequencies in the two modes of sexual reproduction, and transient chromosomal loss and duplication likely occurs frequently and results in aneuploidy and loss of heterozygosity that can span entire chromosomes. Our results thus provide definitive evidence that α-α unisexual reproduction is a meiotic process similar to **a**-α bisexual reproduction. The similarity in meiosis is also reflected by the fact that phenotypic segregation for several virulence related traits, including high temperature sensitivity/tolerance, melanin production, as well as resistance to a variety of antifungal drugs, is also similar between the two modes of sexual reproduction. Our ongoing efforts involving whole genome sequencing of a large collection of haploid meiotic progeny, together with phenotyping and QTL analysis of virulence related traits, will help us elucidate the underlying genetic architectures of these key virulence attributes.

128. Identification of NoxD/Pro41 as the homologue of the p22 NADPH oxidase subunit in fungi. Isabelle Lacaze[1], Hervé Lalucque[1], Ulrike Siegmund[2], Philippe Silar[1], Sylvain Brun[1]. 1) LIED-UMR 8236, Univ Paris-Diderot, Paris, France; 2) Institut für Biologie und Biotechnologie der Pflanzen, Westfälische Wilhelms Universität, Schlossplatz 8, D-48143 Münster, Germany.

NADPH oxidases (Nox) are membrane complexes that produce O_2^- by transferring electrons from cytosolic NADPH to oxygen. In Eukaryotes, Nox are involved in major processes like cell proliferation, differentiation and defense. In mammals, O_2^- production by Nox2 relies on the activation of the membrane flavocytochrome b_{558} composed of the catalytic subunit Nox2/gp91 and of p22 by a cytosolic complex composed of p67, p47, p40 and the small GTPase Rac. In fungi, although the composition of the activating complex (p67/NoxR, Rac, Cdc24 and Bem1) is well conserved, the apparent lack of a homologue of *p22* in genomes questioned how the flavocytochrome forms at membranes. Three Nox isoforms are present in the ascomycetes model *Podospora anserina*: PaNox1, PaNox2 and PaNox3. PaNox1 is involved in fruiting body formation, anastomosis, defense, approssorium-like development and Crippled Growth. Remarkably, all these processes are impaired in the *IDC509* mutant strain as in *IDC343*, the *PaNox1* mutant strain. We have identified *Pa_1_7250* as the gene mutated in the *IDC509* strain. By careful phylogenetic and functional analyses, we show that *Pa_1_7250*, which is homologous *to SmPro41* of *Sordaria macrospora*, is the orthologue of mammalian *p22*. We therefore named it *NoxD* for *Nox Docking protein*. While the physical interaction between NoxD and Nox1 was demonstrated in the plant pathogen *Botrytis cinerea* by Ulrike

Siegmund and collaborators in Paul Tudzynski's lab, our cytological analyses of functional tagged versions of PaNox1, PaNoxD and PaNoxR show that they co-localize in the endoplasmic reticulum and/or in the vacuolar system suggesting the assembly of an active Nox complex.

129. A 'developmental hourglass' in mushroom-forming fungi. X. Cheng, J.H.L. Hui, Y.Y. Lee, M.C. Wong, H.S. Kwan. School of Life Sciences, The Chinese University of Hong Kong, New Territory, Hong Kong.

The 'developmental hourglass' concept suggests that vertebrates are most similar to one another during mid-embryogenesis, and this highly conserved stage is illustrated by the 'waist' of the hourglass representing a low probability of evolutionary change. Recent molecular surveys on both animals and plants have shown that the genes expressed at the waist stage are more ancient and more conserved in their expression. The existence of such a developmental hourglass has not been explored in fungus, a eukaryotic kingdom that evolved with multicellularity. In this study, we analyzed two series of whole-genome expression data on mushroom development – a microarray assay on *Coprinopsis cinerea* and an RNA-seq profiling on *Lentinula edodes*. We found that both mushrooms display a molecular hourglass pattern over their developmental lifecycles. The 'young fruiting body' (YFB) is the stage that expresses the evolutionarily oldest (lowest transcriptome age index, TAI) transcriptome. To put the developmental pattern into functional context of mushroom development, we categorized expressed genes into Eukaryotic Orthologous Groups (KOG). We found that the expression of genes in 'information storage and processing' reached a maximum at YFB and decreased later at the MFB, while genes in 'metabolism' was the lowest at YFB. The synchronic existence of a molecular 'hourglass' across species reveals a common strategy used by eukaryotes to incorporate evolutionary innovations.

130. RNA editing regulates the reproductive cycle in the apomictic fungus *Arnium arizonense* (*Podospora arizonensis*). E. Coppin[1], J. Ait Benkhali[1], D. Zickler[1], C. Drevet[2], A. E. Bell[3], D. P. Mahoney[3], R. Debuchy[1]. 1) Univ Paris-Sud, Inst de Genetique/Microbiology, Orsay, France; 2) Univ Paris-Sud, eBio bioinformatics plateform, Orsay, France; 3) Private Mycological Research, 45 Gurney Road, Lower Hutt, New Zealand.

The ascomycete *Arnium arizonense* is apomictic, *i.e*. dikaryotic croziers are formed inside the fruit-bodies but neither karyogamy nor meiosis take place in the asci. Instead of meiosis, the two nuclei undergo two mitoses and the resulting eight nuclei are enclosed in uninucleate ascospores. We investigated the mating-type structure and function in this species that produces offsprings genetically identical to the parent. *A. arizonense* is closely related to the heterothallic *Podospora anserina* but its mating-type locus displayed a typical homothallic structure consisting of linked counterparts of the *P. anserina* mating-type genes. Deletion of the mating-type locus resulted in the absence of perithecia, thus confirming the critical role of the mating-type genes in the apomictic reproductive cycle. The *AaSMR2* (*MAT1-1-3*) gene harbours a +1 frameshift insertion in the region of the HMG-box, suggesting that *AaSMR2* may be a pseudogene. The degenerescence of *MAT1-1-3* gene is fairly common in homothallics. However, deletion of the *AaSMR2* gene resulted also in the absence of perithecia, indicating that *AaSMR2* is not a pseudogene. Analysis of the *AaSMR2* transcripts revealed that they consist of two categories, one corresponding to the gene sequence while the other category is missing the +1 frameshift mutation in the region of the HMG-box. The absence of any *AaSMR2* gene corresponding to the latter sequence in the genome of *A. arizonense* indicates that these messengers result from the editing of the *AaSMR2* transcripts. Transformation of the *ΔAaSMR2* strain with a gene missing the +1 frameshift insertion restored the formation of perithecia, confirming the critical role of this gene and of editing for the reproductive cycle of *A. arizonense*. Editing was not detected in vegetative phase, indicating that this event was developmentally regulated. A model for the role of editing in the conversion of the homothallic *A. arizonense* in a functional heterothallic will be presented.

131. Characterization of some developmental regulators in the mushroom *Coprinopsis cinerea*. W. Khonsuntia, B. Dörnte, U. Kües. Dept Molec Wood Biotech, Univ Goettingen, Goettingen, Germany.

Three putative genes involved in developmental processes in *Coprinopsis cinerea*, *crg1* and two *flu1-II* copies, are being investigated. The homologous genes in the ascomycete *Aspergillus nidulans flbA* and *fluG* work mutually to activate the process of conidiation by initiation of *brlA* expression. Inactivation of these two genes in *A. nidulans* results in fluffy colony growth [1]. FluG contains an N-terminal amidohydrolase domain and a C-terminal glutamine synthase I (GSI)-like domain and activates conidiation as a specific developmental pathway. For function as a regulator, the GSI-like domain is essential but it does not confer glutamine synthase activity [2]. The two *fluG*-homologs in *C. cinerea* contain the C-terminal GSI-like domain but not an amidohydrolase domain. Crg1 as FlbA homolog possesses two DEP (Dishevelled, Egl-10, and Pleckstrin) domains which function in subcellular targeting and as a C-terminal RGS (regulator of G protein signaling) domain. *crg1* homologs in other fungi take part in regulation of processes such as vegetative growth, asexual sporulation, mating, mycotoxin and pigment production and pathogenicity [3]. Genes *crg1* and *crg2* in the basidiomycetous yeast *Cryptococcus neoformans* and the homologous gene *thn1* in the filamentous *Schizophyllum commune* are functionally coupled to G-protein signaling in the pheromone- and the cAMP-response pathways [4,5]. In *C. cinerea*, we are investigating the functions of *crg1* and *fluG* in vegetative growth of mono- and dikaryons, in asexual sporulation (oidiation), in mating and in fruiting body formation. To examine the functions of *crg1* and *flu1-II* genes, we approach overexpression of the proteins as well as knocking out the genes in suitable strains with a *ku70* mutation [6], with and without activated mating type pathways.

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132. Structural basis for Mep2 ammonium transporter channel opening by phosphorylation. Julian Rutherford, Anupama Chembath, Bert Van-Den-Berg. Institute for Cell and Molecular Biosciences, Newcastle University, UK.

Fungi use ammonium as a nitrogen source to synthesis essential metabolites. Fungal ammonium import is mediated by transporters belonging to the conserved AmtB/Mep2 family of proteins that are found in bacteria, fungi and plants. Fungi typically contain two or more Mep proteins and, of these, orthologues of Mep2 from *Saccharomyces cerevisiae* are of special interest as they act as ammonium sensors that regulate morphological change. Although solved structures of bacterial AmtB transporters have been characterized no structural information is available for the fungal Mep2 proteins. Here we present the X-ray crystal structures of the Mep2 transporters from *S. cerevisiae* and *Candida albicans* using data to 3.2 Å and 1.5 Å respectively. The structures of ScMep2 and CaMep2 are similar and share common features with bacterial AmtB proteins. There are, however, considerable differences between the fungal and bacterial proteins. Notably, the fungal channel is closed on the intracellular side due to movement of the C-terminal region (CTR) and intracellular loops IL1 and IL3 relative to the bacterial proteins. Mutation of the larger fungal CTR to mimic phosphorylation by the TOR effector kinase Npr1 results in a large conformational change due to the formation of a 12 residue long a-helix in the CTR enhancer domain. This is the first conformational change observed in an ammonium transporter as the result of a defined mutation. We propose that under nitrogen–sufficient conditions Mep2 is in a non-phosphorylated state and closed. In response to limiting nitrogen, Npr1 dependent phosphorylation of the Mep2 CTR causes a conformational change in the CTR that brings it into contact with IL1 and IL3 to facilitate pore opening.

133. *Agrocybe aegerita* – a potential model organism for the genetics of basidiocarp development. R. Herzog[1,4], D. K. Gupta[1,2,4], R. Sharma[1,2,4], M. Rühl[3,4], M. Thines[1,2,4], F. Hennicke[1,4]. 1) Goethe-University Frankfurt, Institute of Ecology, Evolution and Diversity, Frankfurt am Main, Germany; 2) Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, Germany; 3) Justus-Liebig-University Giessen, Institute of Food Chemistry and Food Biotechnology, Giessen, Germany; 4) LOEWE Excellence Cluster for Integrative Fungal Research (IPF), Frankfurt am Main, Germany.

The research on model organisms is important for the optimization of strains and breeding strategies to increase fruiting body yield and quality in edible mushroom production. Still, scientific findings with single models are of limited representativeness, especially for commercially interesting mushrooms, and with some of the few current models molecular studies are difficult. Here, we propose the industrially cultivated basidiomycete *Agrocybe aegerita* as a promising candidate for a new model agaricomycete largely combining advantageous features from established models. A suitable dikaryotic strain was selected by the criteria of being easy to cultivate, exhibiting a short life cycle including fruiting and asexual sporulation under axenic conditions, being sensitive to dominant selection markers plus being generally accessible for transformation, having an acceptable genome size for whole genome sequencing and a good yield of aromatic fruiting bodies when grown on different substrates. The selected strain, *A. aegerita* AAE-3, complies with these criteria. AAE-3 exhibits a short life cycle of 21 days on agar media. At the same time, AAE-3 is sensitive to widely-used dominant selection markers. Forty monokaryotic strains were generated from AAE-3. Among them, all published monokaryotic fruiting types of *A. aegerita* could be observed. One mating compatible pair of monokarya was selected for their abundant oidia formation potentially useful for transformation. The whole genome of AAE-3 has been sequenced and is in the process of being annotated. These findings together with the general accessibility of *A. aegerita* to transformation, and its excellent basidiocarp aroma profile make this fungus a promising candidate for becoming a new model agaricomycete.

134. CO_2 repression of fruiting body formation in *Schizophyllum commune* is mediated via cAMP. Jordi Pelkmans, Sanne Westhoff, Han Wösten, Luis Lugones. Microbiology, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands.

The most important environmental cues impacting fructification in *Schizophyllum commune* are light and CO_2. Blue light is needed to initiate fructification, while high levels of CO_2 repress this process. The most ubiquitous mechanism of CO_2 sensing in prokaryotes and eukaryotes is based on cellular levels of cAMP. Regulation occurs through synthesis and degradation by adenylate cyclase (AC) and phosphodiestherase (PDE2), respectively. Most ACs localize to membranes and are activated by G proteins. However, there are also soluble ACs that are activated by bicarbonate or calcium ions. It has been proposed that fungal ACs are a hybrid between these two types and in most fungi there is only one gene for this protein. We here studied the mechanism for CO_2 sensing in *S. commune*. Addition of cAMP to the medium inhibited fructification but did not impact growth. Inhibition of fruiting body formation could be counteracted by overexpressing *pde2*. The *pde2* overexpressor also fructified under repressing CO_2 levels where the wild type strain could not, reinforcing the existence of a link between CO_2 and cAMP levels. Addition of IBMX, an inhibitor of PDE2, to the medium abolished the effect of *pde2* overexpression. Taken together, these results show that CO_2 levels are sensed via cAMP.

135. Subcellular reorganization during trichothecene mycotoxin induction in *Fusarium graminearum*. Marike Boenisch, Karen Broz, H.Corby Kistler. USDA ARS Cereal Disease Laboratory and University of Minnesota, 1551 Lindig Street, St. Paul, MN 55108, USA.

The ascomycete fungus *Fusarium graminearum* causes disease on wheat and barley and contaminates grain with trichothecene mycotoxins making it unfit for human consumption. Little is known about cellular and subcellular changes that occur during toxigenesis that may facilitate trichothecene synthesis and export. Recently, we demonstrated that three enzymes catalyzing early and late steps in trichothecene biosynthesis, hydroxymethylglutaryl CoA reductase (Hmr1p), trichodiene oxygenase (Tri4p), and calonectrin oxygenase (Tri1p), localize to spherical subcellular structures called “toxisomes” when grown in toxin inducing medium. The current study revealed that toxisomes also can be observed *in planta* during infection of wheat husks. Inoculation of paleas and glumes with conidia of a Tri4p::RFP strain reveal toxisomes in infection cushions and runner hyphae. *In vitro*, toxisomes co-localize with the endoplasmic reticulum (ER) as determined by using the fluorescent dye ER-Tracker Blue-White DPX. ER organization shifts from being highly reticulate under toxin non-inducing conditions to being tubular and with pronounced perinuclear ER upon toxin induction. Both ER-Tracker and Hmr1p::GFP fluorescence show similar reorganization during toxin induction. The spherical toxisomes surround nuclei as determined using a Tri4p::RFP/ histone H4p::GFP tagged strain. Thus, toxisomes appear to be perinuclear ER remodeled under toxin inducing conditions. For all three tagged enzymes examined, fluorescent patches co-localizing with ER-Tracker but not associated to nuclei are also

visible under toxin induction. As a consequence, trichothecenes may be produced at both perinuclear and peripheral ER. In contrast to the biosynthetic enzymes, the export of trichothecenes is linked to endosomes. The trichothecene transporter Tri12p tagged with GFP localizes to motile vesicles, vacuoles and the plasma membrane based on co-localization with the fluorescent dyes CMAC and FM4-64. The biosynthesis and transport of trichothecenes in cellular compartments may play a role in sequestration of trichothecene molecules within the cell and self-protection from the toxin.

136. Exploring the role of intracellular pH dynamics in signalling and virulence of *Fusarium oxysporum* using the ratiometric pH sensor pHluorin. Tânia Ribeiro Fernandes, Antonio Serrano, David Turrà, David Segorbe, Antonio Di Pietro. Department of Genetics, University of Cordoba, Cordoba, Spain.

Ambient pH regulates fundamental processes in fungi such as cell growth, development, metabolism and virulence. We previously showed that extracellular pH governs infectious growth in the plant pathogen *Fusarium oxysporum* by reprogramming phosphorylation levels of distinct mitogen-activated protein kinases (MAPKs). The molecular events underlying this response are currently unknown. Here we investigated the hypothesis that intracellular pH (pH_i) acts as a second messenger to transmit environmental cues to the cell signalling machinery. To this aim, a reporter system was established that allows monitoring of pH_i dynamics in living cells by means of the ratiometric GFP-based pH sensor pHluorin. We found that *F. oxysporum* responds to extracellular alkalinization and acidification with a transitory shift in pH_i. Interestingly, addition of nutritional signals such as glucose or ammonium also induced rapid fluctuations in pH_i. To genetically manipulate pH_i homeostasis in *F. oxysporum*, we generated mutants in the pH response elements PalH and PacC, the plasma membrane P-type H^+-ATPase Pma1, its upstream activating protein kinase Ptk2, and the vacuolar proton-translocating ATPase. Labelling of these mutants with pHluorin is being carried out to dissect the role of pH_i in fungal signal transduction and virulence..

137. Four color imagining by fluorescent protein tagging system in *Neurospora crassa*. Ayumi Yokoyama, Miki Uesaka, Shinji Honda. Life Science Unit, University of Fukui, Eiheiji, Fukui, Japan.

Fluorescent proteins are powerful tools to monitor the dynamic localization of proteins of interest. Since the initial report of utility of green fluorescent protein (GFP) in 1994, many new and improved fluorescent proteins have been generated. Previously, we reported the utility of a series of knock-in vectors containing epitope tags including GFP in Neurospora (Honda & Selker, *Genetics*, 2009). Here we show the construction of blue, red, and near-infrared fluorescent protein tagging system in addition to improved GFPs. Neurospora has a genome defense system, RIP, which detects and mutates duplicate sequences over 80% similarity. To prevent RIP by simultaneous usage of GFP and its derivatives, several of them are codon-optimized in Neurospora. Using the constructed system, we successfully visualized nuclear envelop, telomeres, heterochromatin and centromeres by blue, green, red and near-infrared fluorescent proteins, respectively.

138. Dynamics of the actin cytoskeleton in *Phytophthora infestans* hyphae and infection structures. Harold J.G. Meijer[1], Kiki Kots[1,2], Chenlei Hua[1], Tijs Ketelaar[2], Francine Govers[1]. 1) Lab. of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Lab. of Cell Biology, Wageningen University, Wageningen, Netherlands.

The actin cytoskeleton is a dynamic but well organized intracellular framework that is indispensable for the viability of eukaryotic cells. Its functions range from intracellular transport, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. We studied the actin cytoskeleton dynamics in the filamentous oomycete plant pathogen *Phytophthora infestans* in transgenic lines expressing the actin binding peptide Lifeact-eGFP by fluorescence microscopy. This showed that in hyphae actin filament cables and plaques are cortically localized. The distance between the hyphal tip and the first actin filament plaque correlated strongly with growth velocity. Upon growth termination, actin filament plaques appeared in the hyphal tip. The plaques were nearly immobile with average lifetimes exceeding one hour; much longer (over 500-fold) than the lifetimes of actin patches in fungi. Plaque assembly required ~30 seconds while disassembly took only ~10 seconds. In contrast to actin patches in yeast, plaque disassembly was not accompanied with formation and internalization of endocytic vesicles (Meijer et al. 2014, Cell. Microbiol.). We also investigated the *in vivo* actin dynamics during early stages of pathogenesis. At the site of contact with the plant cell a condensed transient actin structure was observed that resembles aster-like actin structures formed upon encountering hard surfaces. Our results suggest that the actin cytoskeleton has distinct functions during the *P. infestans* lifecycle. Future efforts will focus at identifying interactors and key regulators of the actin cytoskeleton and pinpoint features in the actin network that are unique for oomycetes.

139. The photoreceptor WcoB accumulates in the cytoplasm and interacts with carotenogenic proteins in *Phycomyces blakesleeanus*. Alejandro Miralles-Durán, M. Antonia Sánchez-Romero, Luis M. Corrochano. Department of Genetics, Univ of Seville, Seville, Spain.

Phycomyces blakesleeanus is sensitive to environmental signals such as light, wind, gravity and pressure. Light modifies the direction of growth of the fruiting body, sporangiophore, (phototropism), stimulates the production of beta-carotene in the mycelium and regulates the development of the sporangiophores. Blue light is sensed through the Mad complex, a transcription factor complex composed of MadA and MadB. MadA and MadB are homologs of WC-1 and WC-2 from *Neurospora crassa*. The *Phycomyces* genome has three genes homologs to *wc-1*: *madA, wcoA* and *wcoB*; and four genes homologs to *wc-2*: *madB, wctB, wctC* and *wctD*. WcoB contains a LOV domain for chromophore binding, but lacks the Zn finger domain. We have characterized the localization of WcoB in the mycelium of *Phycomyces* using an antibody raised against a peptide of WcoB. The gene *wcoB* is induced by light in vegetative mycelia, but WcoB was present in mycelia kept in the dark or exposed to light. The induction by light of transcription did not result in a major change in the amount of WcoB. In order to identify the cellular localization of WcoB we performed cellular fractionations using cultures grown in the dark or exposed to 30 min of light. We detected WcoB in the cytoplasmic fraction of cellular extracts, while the nuclear fraction was devoid of WcoB. Immunofluorescence assays with spores or germinating mycelia showed that WcoB was detected as localized patches in the cell membrane. Our results suggest that WcoB does not act as a transcription factor and is located in the cell membrane. In order to identify proteins that interact with WcoB we performed immunoprecipitation assays. Candidate proteins were excised from the electrophoresis gel

and sequenced. Several proteins were immunoprecipitated with WcoB: HMG-CoA, CarRA, CarB and CarS. These proteins participate in the biosynthesis of beta-carotenes and the trisporic acid pheromones. Our results suggest that WcoB is a photoreceptor for the regulation of the enzymes that that participate in the biosynthesis of beta-carotene and pheromones.

140. Investigating the role of chitin deacetylation in the rice blast fungus, *Magnaporthe oryzae*. Ivey Geoghegan[1], Sarah Gurr[2]. 1) Department of Plant Sciences, University of Oxford, United Kingdom; 2) Biosciences, Geoffrey Pope Building, University of Exeter, United Kingdom.

The fungal cell wall is a complex and dynamic structure. Alterations in cell wall composition can lead to significant changes in the physical and chemical properties of the cell wall. Such changes are vital in allowing fungi to maintain cellular integrity in the face of challenging environmental conditions, as well as during cellular morphogenesis. Chitin, a polymer of N-acetylglucosamine, is known to be deacetylated in some fungi at specific stages in the lifecycle. In this study, the role of chitin deacetylation in the rice blast fungus *Magnaporthe oryzae* was investigated.

Single, double and triple knockouts of chitin deacetylases were generated, and vegetative growth, conidiation and pathogenicity assayed, comparing wild type and mutant strains. These analyses revealed that chitin deacetylation occurs at many stages in the life-cycle of *M.oryzae*, suggesting that these proteins play broad roles in fungal growth and development.

Various fluorescent protein fusions revealed the cellular localization of the chitin deacetylases and provided further evidence for the multifaceted roles of these enzymes. Co-localization of chitin synthases and chitin deacetylases suggest that these proteins may act in concert.

141. Investigating the role of BAR domain proteins during plant infection by *Magnaporthe oryzae*. Magdalena Martin-Urdiroz, Martin J Egan, Miriam Oses-Ruiz, Darren Soanes, Nicholas J Talbot. School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, England, EX4 4QD, United Kingdom.

The blast fungus *Magnaporthe oryzae* causes a serious disease on a wide variety of grasses including rice. The infection process is initiated when a spore lands on the surface of a leaf and attaches tightly to the cuticle. Then, the spore forms a polarized germ tube, which extends before swelling at its tip, changing direction and becoming flattened against the surface. This process constitutes a recognition phase, which precedes development of a specialized infection cell, the appressorium. This dome-shaped cell generates enormous turgor pressure, which is translated into physical force at its base to rupture the rice leaf cuticle using a narrow, rigid, penetration peg that invades plant tissue. In order to elaborate a penetration peg *M. oryzae* must regulate membrane curvature at the appressorium pore ahead of rapid cell wall biogenesis and apical growth. The generation of concave or convex curvature at membranes is thought to be induced by a variety of protein-driven mechanisms, including the action of BAR domain family proteins. In *M. oryzae*, seventeen BAR domain proteins have been identified. Atg24 (Snx4) and Snx41 are involved in mitophagy and pexophagy and endosomal sorting, respectively, but the specific function of the other BAR domain proteins is unknown. We are currently characterising homologs of Rvs167, Rvs161, KAR3, Cdc24, Snx4, Gvp36, Vps17, Vps5, Snx41, Rgd1, Rgd2, Bzz1, Hof1, Pil1, Lsp1 and SIP3. We have shown that Pil1 and Lsp1 constitute an eisosome, which we have shown is involved in endocytosis and plays roles in conidial germination and germ tube development. We have also found that Rvs167-GFP localizes to the centre of the appressorium pore prior to emergence of the penetration peg. *RVS167*, *ATG24* and *HOF1* are all differentially expressed during appressorium development, while expression of the genes *Lsp1*, *Pil1*, *Rgd1* and *Rgd2* is low. Here we report the localisation and functional analysis of BAR domain proteins in *M. oryzae* and, in particular, explore their role in septin-mediated appressorium penetration and the formation of penetration hyphae during plant infection.

142. Identification and characterization of a gene encoding a dual-specificity tyrosine-regulated protein kinase in the rice blast fungus *Magnaporthe oryzae*. Jong-Hwan Shin, Joon-Hee Han, Kyoung Su Kim. Kangwon National University, ChunCheon, Kangwon, South Korea.

Magnaporthe oryzae is one of the most destructive fungal pathogens causing the rice blast disease. Conidiation is a key process for polycyclic dissemination of *M. oryzae*. To understand the molecular events during conidiation of *M. oryzae*, we performed microarray analysis. Among the genes that are found to be up-regulated during conidiation, we first selected MGG_06399 encoding a dual-specificity tyrosine-regulated protein kinase (DYRK), homologous to YAK1 in yeast. By the homology dependent gene replacement, we made a *ΔMoyak1* mutant. The *ΔMoyak1* mutant showed a remarkable reduction in aerial hyphal formation, cell surface hydrophobicity, and conidiation. The conidia produced by the *ΔMoyak1* were abnormally shaped, smaller than wild type, and showed lower glycogen content than wild type. The conidia were delayed in germination and failed to form appressoria on a hydrophobic coverslip, but formed appressoria with an exogenous cAMP treatment. Moreover, the *ΔMoyak1* mutant was completely defective in pathogenicity on rice. These data indicate that *M. oryzae* Yak1 is associated with cAMP/PKA pathway and important for hydrophobicity, conidiation, condial morphology, conidial adhesion, conidial germination, appressorial formation, and pathogenic development in *Magnaporthe oryzae.*

143. The MoSec4 Protein Is Essential for the Vegetative Development and Pathogenicity by Regulating the Secretion of Extracellular Proteins in *Magnaporthe oryzae*#. Huakun Zheng[1,2], Simiao Chen[1], Xiaofeng Chen[1], Shuyan Liu[1], Xie Dang[1], Guangpu Li[3], Zonghua Wang[1], Barbara Valent*[2], Jie Zhou*[1]. 1) Key Laboratory of Biopesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou 350002, China; 2) Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; 3) Department of Biochemistry and Molecular Biology The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA.

The Rab/GTPases play important roles in the development and pathogenicity of fungus. Recently, the exocyst complex components were

proved to participate in the secretion of cytoplasmic effectors in the rice blast fungus *Magnaporthe oryzae*. To explore the functions of the exocyst complex components in the pathogenicity of the blast fungus, we knocked out the homolog of yeast Sec4p protein, namely MoSec4, in *M. oryzae*. The *Δsec4* mutant is defective in polarized growth and conidiation, and displays decreased appressorium turgor pressure, inhibited secretion of extracellular enzyme and attenuated pathogenicity on rice. We are performing live cell imaging of mutant strains secreting cytoplasmic effector fusion protein PWL2-mCherry-NLS and apoplastic effector fusion BAS4-GFP during biotrophic invasion. Secretion of PWL2-mCherry-NLS into the Biotrophic Interfacial Complex appears partially disrupted in the *Δsec4* mutant, whereas secretion of Bas4-GFP appears normal. Our results suggest that the MoSec4 protein plays an important role in the secretion of extracellular proteins, including the cytoplasmic effectors and consequently the vegetative development and pathogenicity in *M. oryzae*.

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144. Early endosomes organize the fungal cell. Gero Steinberg. Sch Biosci, Univ Exeter, Exeter, United Kingdom.

Fungal early endosomes are a highly motile compartment. The individual organelles travel over long distances along microtubules and extensive work in the basidomycete *Ustilago maydis* and the ascomycete *Aspergillus nidulans* has demonstrated that the molecular machinery, underlying this process is conserved amongst the filamentous fungi (overview in Steinberg, *Curr Opin Microbiol*, 2014, 20:55). However, the biological reason for early endosome motility is largely unknown. Recent papers have revealed unexpected roles in RNA and polysome motility and a have shown a pivotal role in triggering effector production during plant infection. In this talk, I will review briefly these data and present novel roles for moving endosomes in organizing the fungal cell. Taken together, it emerges that early endosomes are of key importance for fungal growth and pathogenicity.

145. A HAD family phosphatase PSR-1 regulates Circadian Output pathway in *Neurospora crassa*. X. Zhou, B. Wang, C. Mallappa, J. Loros, J. Dunlap. Department of Genetics and Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH03755, USA.

Circadian clocks are ubiquitous in eukaryotic organisms where they are used to anticipate regularly occurring diurnal and seasonal environmental changes. *Neurospora crassa* has been used as a principal model organism for studying the circadian system for more than half a century. Nevertheless, little is known regarding pathways connecting the core clock to its output pathways. Here, we demonstrate a HAD family phosphatase PSR-1 is involved in circadian clock output. The *psr-1* deletion mutant has a circadian output defect on race tubes under free running conditions; constant conidiation is observed instead of rhythmic banding. However, further analysis indicates that the FRQ-WCC (FREQUENCY-WHITE COLLAR COMPLEX) oscillator functions normally in *Δpsr*-1 strains although with a three-hour phase delay. PSR-1 is important for maintaining WC-1 (WHITE COLLAR-1) protein and phosphorylation levels and for the interaction between VVD (VIVID) and WC-1. Increased WC-1 protein amounts can partially rescue the phase delay phenotype seen in the *Δpsr-1* mutant. Protein purification of PSR-1 shows it is part of a PSR-1/WHI-2 complex and that *Δ*whi-*2* has phenotypes similar to those observed for *Δpsr-1*. Together, our findings suggest that the PSR-1/WHI-2 complex participates in *Neurospora* clock function by regulating WC-1 levels.

146. The role of calcium signalling in the mode of action of PAF26. Akira JT Alexander, Nick D Read. MFIG, University of Manchester, Manchester, United Kingdom.

Fungal spores are present in the air in high numbers, even in apparently clean environments. In healthy people these present no risk, as the immune system is well adapted to responding. The ability to extend the lives of immunocompromised HIV sufferers and the use of immunosuppressant drugs and broad-spectrum antimicrobials during medical treatments has opened a new niche for opportunistic fungal species. Invasive fungal infections can have very high mortality rates.

Due to the similarity between fungal and mammalian systems, it has been problematic to design highly selective, targeted medications to treat invasive fungal diseases. Current treatments are often harsh with relatively high toxicity levels. The discovery of small, artificially created peptides with antifungal action has the potential to treat emerging fungal pathogens with minimal adverse side effects. A hexapeptide termed Peptide Anti Fungal 26 (PAF26) has been shown to be highly effective at killing filamentous fungi whilst showing low toxicity to bacteria and human cells.

Unlike membrane permeabilising antimicrobials, PAF26 is actively taken up by fungal cells at low fungicidal concentrations and causes cell death through mechanisms not yet understood. There is a dose dependent increase in cytosolic free calcium after treatment, which correlates with peptide internalisation into vacuoles, vacuolar expansion, active export into the cytoplasm and subsequent cell death. Calcium signalling is ubiquitous among eukaryotes, and fungal cells are highly sensitive to disruption of their calcium homeostasis. Deletion of various components of the calcium signalling machinery was found to increase resistance to PAF26 in the model fungus *Neurospora crassa*. The passage of the peptide from the cell exterior up until cell death was blocked at various key stages in these mutants providing evidence particularly for calcium signalling playing a role in the processes of internalisation and vacuolar fusion. The calcium signalling machinery of fungi is sufficiently different from other eukaryotes as be potentially targeted in a fungal specific manner by novel antifungal agents.

147. Assessing the role of exocytosis and endocytosis in fungal morphogenesis. Salomon Bartnicki-Garcia, Fernando Lara-Rojas, Rosa Mourino-Perez. Dept Microbiology, CICESE, Ensenada, Mexico.

The involvement of vesicles in the growth of fungal hyphae is well established but the complexity of the process leaves many unanswered questions. Whereas exocytosis is directly responsible for the growth of the cell wall and plasma membrane, the exact role of endocytosis has yet to be clearly defined. It is commonly assumed that exocytosis creates an excess of plasma membrane and thus the need for removal by endocytosis. This excess seems to be caused by the greater number of vesicles required for cell wall extension and extracellular enzyme secretion over the amount necessary for just plasma membrane extension. The highly localized processes of exocytosis (hyphal apex) and endocytosis (subapical collar) can be seen in living cells by following actin dynamics with Lifeact tagged hyphae. Estimates of membrane flow between exocytosis and endocytosis are difficult to calculate given the absence of reliable values for some critical parameters. Nevertheless, we have developed an interacting spreadsheet to examine the interplay of parameters for which actual data exist such as growth rate, cell shape and size, wall thickness and vesicle size. But in the absence of factual data for other critical factors such as amount of wall generated by each exocytic discharge, relative contribution of macro- vs. microvesicles, proportion of pre-formed cell wall vs. polymer synthesized in situ, and vesicle load destined for extracellular secretion vs wall formation, we have embodied them into a single factor: "vesicle packing efficiency". Accordingly, using the best estimates for critical parameters, an excess of plasma membrane was always produced from exocytosis in a simulated hypha of *Neurospora crassa*. Experimental measurements of endocytosis were attempted by photobleaching the subapical endocytic collar of hyphae of *N. crassa* tagged with endocytic reporters fimbrin-GFP or coronin-GFP. The transient appearance of fluorescent patches, each indicative of an endocytic event, was monitored by confocal microscopy. Based on an estimated range of 50 - 80 nm for the diameter of endocytic vesicles, we recorded values indicating that 5 to 19% of the exocytosed membrane was endocytosed.

148. A role for VE-1 in light sensing and conidal development in *Neurospora crassa*. M. M. Gil-Sánchez, E. M. Luque, L. M. Corrochano. Dept Genetics, Univ Seville, Seville, Spain.

Neurospora crassa is a model for fungal photobiology. Light is perceived through the WC complex, a light-dependent transcription factor complex that regulates transcription. In addition, the *N. crassa* genome contains genes for secondary photoreceptors: two phytochromes genes (*phy-1* and *phy-2*), one cryptochrome gene (*cry-1*), and one opsin gene (*nop-1*). In addition, the *N. crassa* genome contains a homolog of the *Aspergillus nidulans veA* gene, *ve-1*. In *A. nidulans* mutations in *veA* results in constitutive conidiation that is independent of light, and the VeA protein forms a complex with blue and red photoreceptors. The *N. crassa ve-1* mutant has defects in aerial hyphal growth and increased conidiation. We have characterized the light-dependent accumulation of carotenoids in strains with deletions in these genes and in the wild-type. The kinetics and sensitivity is not altered in the *phy-1* mutant. However, a reduction in the maximum accumulation of carotenoids was observed in strains with mutations in *phy-2*, *nop-1* and *cry-1*. A ten-fold reduction in sensitivity was observed in the strain with a mutation in *ve-1*, an indication for a role of VE-1 in light sensing in *N. crassa.* VE-1 is a protein with a nuclear localization signal and a velvet factor domain that is highly conserved in fungi. We observed a minor increase in the accumulation of *ve-1* mRNA after light exposure in vegetative mycelia (30 min), which did not lead to a major change in VE-1 accumulation. The mutation in *ve-1* results in decreased light-dependent accumulation of mRNA for several genes, including the carotenogenesis genes (*al-1, al-2, al-3, cao-2*), *wc-1*, *vvd*, and *frq*. We have characterized the cellular localization of VE-1 under different light conditions and we have observed that VE-1 is preferentialy located in the nucleus under all conditions, but VE-1 was also detected in the cytoplasm. We detected VE-1 in vegetative mycelia in the dark but light promoted the accumulation of VE-1 in vegetative mycelia, aerial hyphae and conidia through the activity of the WC complex. The light-dependent accumulation of VE-1 in aerial hyphae and conidia suggests a role for this protein in conidial development.

149. Biological Significance of Photoreceptor Photocycle Length: role of VIVID photocycle in establishing a dynamic VIVID-White Collar Complex pool. Arko Dasgupta[1], Chen-Hui Chen[1], ChangHwan Lee[2], Amy Gladfelter[2], Jay Dunlap[1], Jennifer Loros[1,3]. 1) Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Biological Sciences, Dartmouth College, Hanover, NH; 3) Department of Biochemistry, Geisel School of Medicine at Dartmouth, NH.

Most organisms on earth sense light through the use of chromophore bearing photoreceptive proteins with distinct and characteristic photocycle lengths, yet the biological significance of photocycle length is neither understood nor been tested. In the filamentous fungus *Neurospora* crassa VIVID (VVD) is a critical player in the process of photoadaptation.Detailed *in vitro* analysis of the photochemistry of the blue light sensing, FAD binding, LOV domain of VVD has revealed residues around the site of photo-adduct formation that influence the stability of the adduct state (light state), that is, altering the photocycle length. We have examined the biological significance of VVD photocycle length to photoadaptation and report that a double substitution mutant (*vvdI74V&I85V*), previously shown to have a very fast light to dark state reversion *in vitro*, shows significantly reduced interaction with the White Collar Complex (WCC) resulting in a substantial photoadaptation defect. This reduced interaction impacts WHITE COLLAR-1 (WC-1) protein stability when *N. crassa* is exposed to constant light with the result that mutant VVD is unable to form a dynamic VVD-WCC pool of the size required for photoadaptation as assayed both by attenuation of gene expression and the ability to respond to increasing light intensity. Additionally, transcription of the clock gene *frequency* (*frq*) is sensitive to changing light intensity in a wild-type strain but not in the fast photo-reversion mutant indicating that the establishment of this dynamic VVD-WCC pool is essential in general light and circadian biology.These data speak to the biological significance of photocycle length: VVD photocycle length appears sculpted to establish a VVD-WCC reservoir of sufficient size to sustain photoadaptation while maintaining sensitivity to changing light intensity. Thus, diversity of photocycle kinetics among photoreceptors may be viewed to have adaptive and functional significance in their native organisms.

150. Characterizing the germling fusion pathway in *Neurospora crassa*. Monika Fischer, Wilfried Jonkers, N. Louise Glass. Plant & Microbial Biology, UC Berkeley, Berkeley, CA.

Establishment of a robust colony is dependent on cell fusion between genetically identical germinating conidia (germlings) in *Neurospora crassa*. Two germlings in close proximity to each other will engage in a molecular conversation that facilitates chemotropic growth, membrane fusion, and cytoplasmic mixing. The conversation is initiated by an unknown factor that leads to activation of a conserved MAP-kinase cascade. Once activated, the MAP-kinase, MAK-2 activates the transcription factor PP-1, which regulates expression of several genes required for homing and fusion. Several genes have been identified as being necessary for germling communication and/or fusion, but most of these genes have remained poorly characterized. We have screened deletion mutants of each of these genes for the output of a communication-activated Luciferase reporter, phosphorylation/activation of MAK-2, and whether or not these phenotypes can be rescued by a constitutively-active upstream MAPKKK (NRC-1). Our data indicates three general groups of genes required for germling fusion; genes that function downstream of the MAK-2 MAPK cascade, genes that function upstream of the MAK-2 MAPK cascade, and genes that are involved in a potential positive feedback loop. Notably we've identified two more transcription factors (ADV-1 and ADA-3) in addition to PP-1 that are required for germling fusion and expression of communication genes.

151. Proteome analysis of transiently oxidized proteins during the hyperoxidant state that triggers conidiation in *Neurospora crassa*. Wilhelm Hansberg[1], Teresa Nava-Ramírez[1], Bastian Jöhnk[2], Oliver Valerius[2], Gehard Braus[2]. 1) Instituto de Fisiologia Celular, Universidad Nacional Autónoma de Mexico, UNAM, Mexico City, Mexico; 2) Institute for Microbiology and Genetics, Georg August University of Göttingen, Göttingen, Germany.

The *Neurospora crassa* asexual cell cycle is stated by filtering an exponentially growing liquid culture and exposing the resulting mycelial mat to the air. The increase in oxygen tension causes oxidative stress in the air-exposed hyphae. Total protein is oxidized during the first 0-10 minutes air exposure followed by protein degradation and resynthesis. Oxidative stress results in hyphal adhesion during the first 40 minutes of air exposure. Using a method to identify proteins with reversible oxidized cysteine residues, we have isolated the cysteine-derivatized proteins at different times of the conidiation process (0, 2, 5, 10, 30 and 60 min air exposure) and identified them by mass spectrometry. A positive control, derivatized proteins from a culture treated 10 min with 20 mM H_2O_2, and a negative control, without derivatizing agent, were included. About 500 proteins were identified that increased in relative amount at 2 - 10 min air exposure. A functional analysis of these proteins will be presented. Results revealed a rapid, transient and extensive response to oxidative stress. Proteins detected are consistent with: growth arrest, unfolded protein response, protein degradation at the ER, vacuole and the proteasome, important mitochondrial and carbon metabolism regulation.

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152. Affinity purification of methyl-lysine proteins in *Neurospora crassa*. Masayuki Kamei, Zachary Lewis. Department of Microbiology, University of Georgia, Athens, GA.

Lysine methylation is thought to be a common post-translational modification that regulates proteins involved in diverse cellular processes. Although lysine methylation of histone proteins has been studied extensively, little is known about non-histone proteins that are regulated by lysine methylation. In addition, there is limited information about the lysine methyltransferases (KMTs) that target non-histone proteins. *Neurospora crassa* has 12 KMT genes. 11 of these encode proteins with a conserved SET domain and 1 is a homolog of DOT1, a conserved non-SET KMT protein. To identify non-histone substrates of KMTs, we carried out a proteomics approach to purify and identify methyl-lysine containing proteins in *Neurospora* cell extracts. Methyl-lysine containing proteins were purified from extracts of wild-type cells and from disruptants of KMT-encoding genes. Our preliminary data suggest that lysine methylation is widespread and impacts diverse cellular processes in fungi, including metabolism, signal transduction, transcription, and RNA degradation.

153. BEM46, eisosomes and auxin biosynthesis in *Neurospora crassa*. Kollath-Leiβ Kriztina, Bönniger Christine, Sardar Puspendu, Frank Kempken. Dept. of Botany, Christian-Albrechts University, Kiel, Germany.

The BEM46 protein is evolutionary conserved in eukaryotes (1). We previously described the BEM46 protein to be targeted to the ER and being essential for ascospore germination in *N. crassa* (2). Upon reinvestigation of the RNAi transformants we found strong evidence for accumulation of alternative spliced mRNAs. One alternative spliced mRNA was found to be 0.5 and the other 1.2 kb in size. Both alternative spliced mRNAs were cloned and expressed in the wild type. Expression led to loss of ascospore germination, indicating that this phenotype is caused by peptides encoded by the alternative spliced mRNAs. Using the *N. crassa* ortholog of the eisosomal protein PILA from *A. nidulans* we demonstrate partial co-localization of BEM46 with eisosomes (3).

Employing the yeast two-hybrid system, a single interaction partner, the anthranilate synthase component two (*trp*-1) was identified, and confirmed *in vivo* by a split-YFP approach. A connection between BEM46 and tryptophan dependent auxine biosynthesis in *N. crassa* was observed. We describe the putative auxin biosynthetic pathway in the fungus using bioinformatical tools (3), and determined the transcription of the involved enzymes in different *bem46* wild type and mutant strains by qRT-PCR approaches. In addition to this the indole production of the strains in different developmental stages was also investigated.

Currently auxin pathway double mutants auxin pathway are under investigation, as well as other components of the Neurospora eisosome.

Ref.: (1) Kumar A, Kollath-Leiß K, Kempken F (2013) *Biochem Biophys Res Comm* 438:526-532 (2) Mercker M, Kollath-Leiß K, Allgaier S, Weiland N, Kempken F (2009) *Curr Genet* 55:151-161 (3) Kollath-Leiß K, Bönniger C, Sardar P, Kempken F (2013) *Euk Cell* 13:1051-1063.

154. The endocytic protein MYO-1 is needed for normal growth and hyphal morphogenesis in *Neurospora crassa*. Fernando Lara, Salomón Bartnicki-García, Rosa Mouriño-Pérez. Departamento de Microbiología, CICESE, Ensenada, B. C., México.

Endocytosis is an important process in eukaryotic cells. Several proteins participate in a coordinated pathway which involves binding membrane receptors, invagination, vesicle formation and scission. In *Neurospora crassa*, the endocytic machinery is located in a sub-apical collar. The proximity of the endocytic sites to the hyphal apex suggests that endocytosis may have a complementary role in apical growth. MYO-1, a class I myosin, is known to play a role in endocytosis. To investigate the possibility that this actin-binding protein is involved in endocytic processes of *Neurospora crassa*, we used deletion mutants together with fluorescent-protein tagging. We found myosin-1-GFP located in the subapical collar in the form of small patches, in partial colocalization with two other endocytic proteins, fimbrin and coronin. Myosin-1 also participates in septum formation as part of the actomyosin contractile ring. The deletion mutant Δ*myo-1* exhibited a severe reduction in growth rate, nearly 95% compared to the wild type strain, irregular branching, reduced aerial mycelium and no conidiation. A reduced uptake of FM4-64 in Δ*myo-1* indicated a partial deficiency in endocytosis. By visualizing actin with the Lifeact-GFP reporter, we found that the absence of *myo-1* altered the actin cytoskeleton, leading to periods of polarized and isotropic growth of the hyphae. Our findings indicate the importance of myosin-1 in the organization and dynamics of the actin cytoskeleton, the stability of the Spitzenkörper, and the maintenance of normal hyphal morphogenesis.

155. Dissecting the genetics of cellulase hypersecretion in *Neurospora crassa*. Jason Liu[1,2], N. Louise Glass[1,2]. 1) Plant and Microbial Biology Department, Univ California, Berkeley, Berkeley, CA; 2) Energy Biosciences Institute, Univ California, Berkeley, Berkeley, CA.

One of the major expenses that govern the price of cellulosic fuels is the cost of the enzymes used to convert lignocellulose into soluble sugars for fermentation. The current industrial cellulase producing host, *Trichoderma reesei*, is able to produce >100g/L of protein, however, the dearth of genetic tools has hindered the identification of synergistic genetic interactions. Utilizing *Neurospora crassa* as a cellulolytic, genetically amendable filamentous fungus, we can dissect the necessary genetic interactions by generating a cellulase hyper-secreting strain through forward mutagenesis and identifying the required mutations. Using a fluorescently labeled cellulase, we are able to visualize cellulolytic induction and sort using fluorescently activated cell sorting (FACS) to isolate mutant strains up regulated in cellulase expression. Subsequently, we use high throughput plate based cellulolytic assays and candidate based approaches to identify strains increased in cellulolytic activity. Through iterative mutagenesis, we have currently isolated two strains that are >8 fold increased in total cellulolytic activity over WT strains. Through iterative backcrossing and bulk segregation analysis, we hope to dissect the genetic interactions necessary for synergistic hyper-secretion of cellulases. With a better understanding of the mutations necessary to form a cellulase hyper-secreting strain in a filamentous fungus, we can apply this knowledge broadly to industrial systems.

156. Role and subcellular localization of EGL-1 and EGL-2, two putative GPI anchored cell wall β (1-3) endoglucanasas, in *Neurospora crassa*. Leonora Martinez, Meritxell Riquelme. CICESE, Ensenada, Mexico.

Hyphal growth presumably involves a balance between synthesis of new cell wall polymers and hydrolysis of pre-existing polymers. EGL-1 (NCU06381) and EGL-2 (NCU09175) are two putative β-(1,3)-endoglucanases in *Neurospora crassa*, with predicted binding sites for glycosylphosphatidylinositol (GPI anchor). Both GFP-tagged proteins were observed at the plasma membrane (PM), forming a collar in the hyphal apical dome. After staining with FM4-64, GFP-tagged proteins were observed excluded from the Spitzenkörper and from the foremost PM apical region, where biosynthetic enzymes have been previously found. EGL-1-GFP and EGL-2-GFP were also observed at the leading edge of a new developing septum, which advanced centripetally until reaching the edge of the septal pore. Both endoglucanases were observed at unreleased interconidial septa. During conidial isotropic growth, the fluorescence, was found all around the conidial surface. EGL-2-GFP fluorescence at interconidial septa was lost in a *N. crassa csp-2*Δ mutant, unable to release conidia from conidiophores. Both tagged proteins were observed at sites of hyphal fusion and emerging branches, confirming a role for these enzymes at sites of cell wall remodeling and at sites of new cell wall synthesis. Single mutant strains *egl-1Δ* and *egl-2Δ* and double mutant *egl-1Δ::egl-2Δ* exhibited a slightly reduced growth rate. Single mutant *egl-1Δ* presented more chains of unreleased conidia and fewer free conidia, while *egl-2Δ* produced both few conidial chains and a significantly lower amount of free conidia, confirming a role for these genes in conidial formation. The double mutant *egl-1Δ::egl-2Δ* produced more conidia than wt. When exposed to calcofluor white and congo red the single mutant *egl-2Δ* and the double mutant *egl-1Δ::egl-2Δ* displayed a higher growth rate than the parental strain. EGL-1 and EGL-2 may have a role in the shortening of preformed glucans. These and previous studies suggest a division of labor during cell wall synthesis at the hyphal dome: at the very tip, glucans are synthesized by enzymes that accumulate at the Spk whereas at the subtending zone below the apex glucans are hydrolyzed, producing amenable ends for further crosslinking.

157. Biogenesis and traffic of CHS-4 in hyphae of *Neurospora crassa*. Adriana Rico Ramirez, Rosa Fajardo-Somera, Meritxell Riquelme. Microbiology, CICESE, Ensenada, Mexico.

The growth of fungal hyphae occurs in the apical region, where addition of new material for expansion of the plasma membrane (PM) and the cell wall, the latter consisting mainly of glucans and chitin, is required. Chitin synthesis is catalyzed by chitin synthases (CHS), a family of PM integral proteins. In *Saccharomyces cerevisiae* Chs3p (class IV CHS) has the most important role in chitin synthesis, since it synthesizes around 90% of the cell wall chitin. Transport of Chs3p requires its interaction with the chaperone Chs7p at the endoplasmic reticulum (ER), and with the exomer complex at the Golgi. The exomer is composed of five subunits, which comprise Chs5p, and four Chs5p-Arf1p binding proteins or ChAPs (Chs6p, Bch2p, Bch1p and Bud7p). Finally, the localization and activation of Chs3p at the PM depends on its interaction with Chs4p. In *Neurospora crassa*, CHS-4, the homologue of Chs3p, is located at the center of the Spitzenkörper (Spk) and in septa in formation. Recent studies suggest that the exocyst tethers the macrovesicles concentrated at the outer layer of the Spk to the regions of the PM, where they will merge. However, there is no evidence so far for the participation of the exocyst or other tethering factor in the fusion of the microvesicles that carry CHS and accumulate at the core of the Spk, with the PM. Homologues of the proteins involved in transport of Chs3p in *S. cerevisiae* have been identified in *N. crassa*: CSE-7 (Chs7p), CBS-5 (Chs5p), BUD-7 (Chs6p, Bch1p,

Bch2p and Bud7p), CSA-1, CSA-2 and CSR-3 (Chs4p). Confocal microscopy analysis of the distribution of CHS-4 tagged with GFP in a *Δcse-7* or *Δbud-7* background showed that the transport of CHS-4 towards the Spk and septa is dependent on CSE-7, but does not depend on BUD-7. In order to assess the involvement of CBS-5, the other putative component of the exomer, as well as the putative orthologues of Chs4p, CSA-1, CSA-2, and CSR-3, and one of the components of the exocyst SEC-5, genetic crosses of strains with corresponding null genetic background for each of these proteins with the CHS-4-GFP expressing strain are being carried out.

158. Protein phosphatase 2A (PP2A), a protein involved in regulation of hyphal elongation in *Neurospora crassa,* interacts with the NDR kinase COT1. H. Shomin, O. Yarden. The Robert H. Smith Faculty of Agriculture, Food and Environment, Department of Plant Pathology and Microbiology. The Hebrew University of Jerusalem, Israel.

The *Neurospora crassa* Nuclear Dbf2-related (NDR) Ser/Thr kinase- COT1 is important for polar growth and normal hyphal development. COT1 regulates elongation and branching in an independent manner which is determined by its phosphorylation state. PP2A is a heterotrimeric Ser/Thr phosphatase that plays an important role in regulation of growth and development in *N. crassa* and other fungi. Reversible methylation of the PP2A catalytic subunit (PP2Ac) has been shown to be involved in the regulation of PP2A holoenzyme assembly in human and yeast and is catalyzed by leucine carboxyl methyltransferase 1/2 (LCMT1/2) and methylesterase 1 (PME1) . Deletion of *pme-1* or *lcm-1* (but not *lcm-2*) in *N. crassa* resulted in increased conidial production and longer distances between hyphal branches. Surprisingly, all double mutant combinations had no synergistic effects on *N. crassa* morphology, suggesting additional, yet unidentified, components may be involved in regulation of PP2A activity. *pph-1*, which encodes PP2Ac, is essential in *N. crassa* and deletion of the PP2A regulatory subunits *rgb-1* and *b-56* impairs hyphal growth, branching and conidiation. On the basis of co-immunoprecipitation experiments we determined that the PP2A catalytic subunit and COT1 can physically interact. We also determined the presence of a genetic interaction between the PP2A regulatory subunit *rgb-1* and *cot-1*(S189E), *cot-1*(S417E) and *cot-1*(T589E), all of which are alleles that mimic constitutive phosphorylation on the respective residues. In all cases, the presence of the phosphomimetic allele suppressed the slow growth phenotype of *rgb-1*^{RIP}, indicating a functional link between the two gene products, on the basis of COT1 phosphorylation state. In contrast, constitutive lack of phosphorylation on S417 suppressed the slow growth of the *b-56* regulatory subunit mutant. We conclude that PP2Ac as well as the RGB1 and B56 regulatory subunits interact with COT1. Based on the differential interactions with *cot-1* phosphomimetic mutants, the regulatory subunits have distinct functions in regulation of the COT1 NDR kinase pathway.

159. Calcium signalling during CAT chemotropisms in *Neurospora crassa*. Patricia Hernandez-Ortiz, Chia-Chen Chang, Meiling Chu, Nick D Read. University of Manchester, Manchester, United Kingdom.

Neurospora crassa undergoes self-fusion as a fundamental biological process required for the formation of an interconnected mycelial network. During colony initiation this process involves the fusion of specialised cell protrusions called conidial anastomosis tube (CAT) derived from conidia and germ tubes. CATs undergo chemotropisms towards each other in a concerted way with the CAT from one cell acting as a 'signal sender' and the CAT from the other as a 'signal receiver'. These signal sending and receiving functions switch back and forth between the CATs growing towards each other (the so-called 'ping-pong mechanism').

Calcium is a ubiquitous signalling molecule which regulates many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, circadian rhythms and virulence. We have found a major role for Ca^{2+} during CAT chemotropism prior to fusion. The absence of extracellular Ca^{2+} inhibited CAT chemoattraction which was restored after subsequent addition of Ca^{2+} to the medium. Moreover, we observed that extracellular Ca^{2+} is required at a very low concentration to allow CATs to undergo fusion. We have also imaged Ca^{2+} dynamics using the genetically encoded Ca^{2+} reporter GCaMP6s. Our results show that CAT chemoattraction does not generate transient increases in cytosolic free Ca^{2+}. The primary intracellular Ca^{2+} receptor, calmodulin (CaM) and its main target protein, the phosphatase calcineurin (CN), also play a role in CAT fusion. CaM antagonists reduced CAT chemotropism while CN antagonists completely inhibited this process. Our data suggests a model in which Ca^{2+} is required extracellularly for the CAT chemoattractant-receptor interaction and components of the intracellular Ca^{2+} signalling machinery are required for the regulation of CAT chemotropic growth.

160. Autophagy but NOT mitophagy elicits lifespan extension of *ClpXP* deletion mutants in the ascomycete *Podospora anserina*. Laura Knuppertz, Fabian Fischer, Heinz D. Osiewacz. Molecular Biosciences, J. W. Goethe-University, Frankfurt, Hesse, Germany.

Mitochondria are eukaryotic organelles involved in various essential functions including iron/sulfur cluster synthesis, lipid and amino acid metabolism, as well as copper homeostasis, and particularly energy transduction. Mitochondrial maintenance crucially depends on the quality control of proteins by various chaperones, proteases and repair enzymes. Failure of these systems leads to perturbations in the normal folding pathways, resulting in the accumulation of misfolded and aggregated proteins that in turn get inoperable or can even compromise the whole cell. The CLPXP protease, localized in the mitochondrial matrix, is a hetero-oligomeric complex of an AAA^+ ATPase (CLPX) and a peptidase (CLPP).

The ascomycete *P. anserina* is characterized by a short lifespan, displays a clear mitochondrial etiology of aging and has been extensively investigated to unravel the molecular basis of aging processes. Unexpectedly, *PaClpP*-, *PaClpX*- and *PaClpXP*- deletion strains are characterized by a healthy phenotype and an increased lifespan. To explain this rather unexpected finding, we investigated of whether or not the longevity phenotype can be reverted by the loss of the autophagic machinery via an additional *PaAtg1* deletion. We further appropriated several reporter strains, like a PaATG8 strain, allowing the microscopic tracking of autophagy, or GFP strains, enabling the measurement of the degradation of GFP fusion proteins via autophagy/mitophagy. These data demonstrate that autophagy is up-regulated and therefor responsible for the lifespan extension in the *PaClpXP* mutants, by "compensating" for the loss of the protease. Surprisingly,

mitophagy appears to be unaffected, suggesting that up-regulation of autophagy but not mitophagy is sufficient to prevent damage accumulation.

161. From two to many: Multiple mating types in basidiomycetes. Ursula Kües. Dept Molec Wood Biotech, Univ Goettingen, Goettingen, Germany.

The tetraploar *Coprinopsis cinerea* has undergone two routes as how to multiply allele numbers of the mating type loci *matA* and *matB*, i.e. by sequence diversification of allelic genes and by generation of sets of paralogous genes with respective distinct alleles. The *matA* locus encodes HD1 and HD2 homeodomain transcription factors in three functional divergently transcribed gene pairs. A compatible interaction is in between an HD1 protein and an HD2 protein from allelic gene pairs. Sequence diversification of the N-termini ensured that the HD1 and HD2 proteins discriminate unwanted proteins from the own gene pair or from paralogous gene pairs from compatible partner proteins from gene pairs of allelic origin. The *matB* locus encodes pheromones and pheromone receptors in at least three functional groups of paralogous genes. Also here, the functional system bases on a lock-and-key-principle which allows compatible interactions only between products from allelic gene groups but not between pheromones and receptors encoded in the same group of genes or in paralogous groups. Additional degenerated genes within the *mat* loci document a history of birth and death of genes in the generation of the larger loci for an optimal number of paralogous genes. Other Agaricomycetes applied similar strategies as *C. cinerea* to multiply mating type specificities. However, gene duplication and paralogous diversification in *matB* is ancient in evolution prior to speciation. Most species have several paralogous genes, even species in which the *matB* control function in mating has been inactivated. Gene duplications in *matA* in contrast are lineage-specific and throughout the Agaricomycetes not as common. Comparison of four *matB* alleles of *Ganoderma lucidum* from the Polypores indicated rearrangements in locus structure after gene duplications so that chromosomal positions of genes of a same origin are not conserved. Also the *matA* region with a single *HD1-HD2* gene pair is not protected in structure. The region underlied rearrangements by transposon insertions and relocation of neighbored genes in some of the genomes to other chromosomal positions.

162. Polar growth and endocytosis in *Ashbya gossypii*. Doris Nordmann[1], Kumiko Masai[2], Peter Philippsen[2], Hans-Peter Schmitz[1]. 1) Department of Biology, University Osnabrück, Germany; 2) Biozentrum, University of Basel, Switzerland.

Polar surface expansion at the tip of hyphae is still poorly understood. Studies in several filamentous fungi have shown a spacial separation of exocytosis at the front of hyphal tips and endocytosis at the rim of hyphal tips. We and others hypothesized that polarity factors, exocyst components, and excess of membranes have to be internalized by endocytosis adjacent to the zone of extensive vesicle fusions in hyphal tips in order to maintain polar growth. We study endocytosis in *A. gossypii* which, based on its genome, is closely related to budding yeast but grows exclusively as multinucleated hyphae with up to 40 times faster surface growth rate compared to budding yeast. A comparative study of endocytic components in both organisms could therefore reveal essential hints to explain the differences in polar growth efficiency. In addition, it is very convenient that actin patches, which mark sites of endocytosis, can be stained in both organisms with Phalloidin, and that targeted gene manipulations work as efficiently in *A. gossypii* as in yeast. We could demonstrate that a streamlined endocytic process is essential for fast polar surface expansion in *A. gossypii*. Hyphal growth rates are decreased in 16 of 20 deletion mutants of endocytic genes. The other four deletions were lethal. The genes were selected from the 4 phases of endocytosis defined in yeast (Kaksonen and Drubin 2003, 2005). Null mutants of the yeast orthologs do not show the same pattern of reduced growth/lethality. In some of the slow growing *A. gossypii* mutants, the zone of endocytosis was no longer localized at the rim of hyphal tips. Next we investigated whether the faster surface growth potential of *A. gossypii* is reflected by faster endocytic events or by a higher density of endocytic events compared to yeast. Using Live-cell imaging Microscopy together with TIRF we monitored 9 fluorescently-labeled endocytic proteins in *A. gossypii* and found that some endocytic steps proceed up to 10 times faster as in yeast. Also, the number of endocytic events per square micrometer is significantly increased in *A. gossypii*. Finally, we tried to find an answer to the question why *A. gossypii* is lacking an ortholog of the important endocytic yeast gene SLA2.

163. *Ustilago maydis* teliospore dormancy is linked with antisense RNAs to nucleus-encoded mitochondrial genes. Lauren Ostrowski[1], Barry Saville[1,2]. 1) Environmental and Life Sciences Graduate program; 2) Forensic Science Program, Trent University, 2140 East Bank Dr., Peterborough, ON, Canada K9J 7B8.

Ustilago maydis is the causal agent of common smut of corn and the model for biotrophic basidiomycete plant pathogenesis. Its development *in planta* during disease formation culminates with the production of thick-walled dormant teliospores. These dispersal agents have very low rates of respiration, indicating that mitochondrial activity is attenuated. One mechanism through which this may be accomplished is by inhibiting the translation of nucleus-encoded mitochondrial mRNAs. Natural antisense transcripts (NATs) are capable of inhibiting translation of their complementary mRNAs. *U. maydis* transcript analysis revealed that as-*ssm1*, a NAT to the mitochondrial seryl-tRNA synthetase (*ssm1*), is preferentially detected in the dormant teliospore and absent in haploid cells. *ssm1* is an essential gene for cell division. We hypothesise that *as-ssm1* has a role in initiating and maintaining cellular dormancy in *U. maydis* through binding to and stabilizing *ssm1* transcripts, thus inhibiting translation and reducing mitochondrial function. During teliospore germination, *ssm1* transcripts would then become available for rapid translation. To assess the impact of *as-ssm1* expression on cell division, pathogenesis and mitochondrial function, *as-ssm1* was ectopically expressed at the *ip* locus in haploid cells. Compared to wild-type haploid cells, these expression mutants demonstrated a significant decline in growth rate in liquid culture, reduction in virulence in seedling pathogenesis assays, and a drop in mitochondrial membrane potential as determined by MitoTracker® Red CM-XRos staining. Further, expression of *as-ssm1* resulted in the formation of double-stranded RNA (dsRNA) with the complementary mRNA in haploid cells, yet *ssm1* mRNA levels remained unchanged. Such dsRNA formation has been shown to inhibit translation of other *U. maydis* mRNAs, and antibodies have been raised to estimate Ssm1 protein levels. Together, these findings support a role for *as-ssm1* in attenuating mitochondrial function during the transition from physiologically active dikaryon filaments to dormant teliospores.

164. Roles of protective pigments in oxidative stress responses of the rock-inhabiting model fungus *Knufia petricola* A95. Nicole Knabe[1], Anna Gorbushina[1,2]. 1) BAM - Federal Institute for Materials Research & Testing, Department 4.1 (Biodeterioration and Reference Organisms) Berlin, Germany; 2) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy & Department of Earth Sciences, Berlin, Germany.

Rock-inhabiting microcolonial fungi (MCF) are able to colonise barren surfaces in almost every environment and are unequalled among eukaryotes in their ability to withstand extreme environmental conditions. Protective pigments, like melanin and carotenoids, have been proven to contribute to this unique robustness. An excellent model system to investigate the involvement of pigments in stress response and DNA repair is the MCF *Knufia petricola* strain A95. This non-pathogenic fungus possesses all characteristic features of MCF, including meristematic growth, melaninised cell-walls and extensive secondary metabolite production. This study is focusing on *K. petricola* responses to oxidative stress - one of the most significant environmental challenges encountered by MCF. The exact role of pigments and especially the interplay between carotenoids and melanin in the oxidative stress response is studied in wild type cells and constructed pigment knock-out mutants. Deletion of polyketide synthase in *K. petricola* (ΔPKS) leads to a complete loss of melanin, phenotypically revealing the carotenoids which are normally hidden beneath the melanised cell wall. In scytalone dehydratase mutants (ΔSDH) the melanin synthesis pathway is not disrupted completely and the colonies appear darker than ΔPKS. Additionally in ΔSDH phenotype precursors of DHN-melanin diffuse into the media causing the reddish-brown staining of the agar. In comparison to the wildtype strain, treatment of both melanin mutant strains with the oxidative agent H_2O_2 (up to 30 mM) shows no dose-dependent growth reduction. This indicates a relevant influence of the carotenoid pigments which has to be proven in further experiments. Comparative gene expression analyses concentrate on genes which are especially regulated under oxidative stress conditions and will help to elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

165. The Oomycete *Phytophthora sojae* uses non-canonical nuclear localization signals to direct proteins into the nucleus. Yufeng Fang[1,2], Brett Tyler[1,2]. 1) Interdisciplinary Ph.D. program in Genetics, Bioinformatics & Computational Biology, Virginia Tech, Blacksburg, VA 24061; 2) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Nuclear localization signals (NLSs) are amino acid sequences that direct proteins from the cytoplasm into the nucleus in eukaryotic cells, which play an important role in regulating nucleo-cytoplasmic transport. However, sequences that determine nuclear localization have not been defined in oomycetes, a fungus-like taxon that belongs to the kingdom *Stramenopila*. In this study we have investigated NLS functioning in *Phytophthora sojae*, a model species of oomycetes that infects soybeans. Using confocal microscopy, we found that two well-studied NLSs, classical NLS (cNLS; from SV40 large T antigen) and proline-tyrosine NLS (PY-NLS; from the acidic M9 domain of hnRNP A1), as well as their derivatives cannot direct fluorescent proteins, such as GFP, into the nuclei of *P. sojae* transformants. Surprisingly, we also found that in highly conserved nuclear-localized proteins, such as ribosomal proteins and core histones, NLSs required for nuclear import in human or yeast, did not function in *P. sojae* even though the sequences were conserved in the *P. sojae* orthologs. Those results suggested that *P. sojae* may use a non-canonical mechanism for nuclear cargo transport. To identify functional NLSs in *P. sojae,* we experimentally examined 20 *P. sojae* nuclear-localized proteins, and defined in detail the NLS in three of those proteins. We found that *P. sojae* NLSs have several unique characteristics: (1) *P. sojae* NLSs are composed of several sub-sequences dispersed across the protein sequence. (2) NLS subsequences can either work individually when present in multiple copies or several subsequences can work collectively to direct proteins into the nucleus. (3) A *P. sojae* NLS was defined as three clusters of four positively charged amino acids with a minimum spacing. When the non-canonical *P. sojae* NLSs were tested in *Arabidopsis* and mammalian cells, they directed nucleolar localization, suggesting that the *P. sojae* NLSs may have evolved from nucleolar localization signals. We discuss the possible significance of this unusual arrangement.

166. Analysis of Septin Organization Using Polarized Fluorescence Microscopy. Molly McQuilken[1], Sara Abrahamsson[2], Shalin B. Mehta[3], Grant Harris[3], Amitabh Verma[3], Rudolf Oldenbourg[3], Amy S. Gladfelter[1]. 1) Biological Sciences, Dartmouth College, Hanover, NH; 2) Lulu and Anthony Wang Laboratory of Neural Circuits and Behavior, Rockefeller University, New York, NY 10065; 3) Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Septins are conserved filament-forming proteins that act in cytokinesis, membrane remodeling, cell polarization, and migration. They closely associate with membranes and help establish dramatic shapes in various fungi. Although septin function is critical for diverse cell events, it is not well understood how they assemble *in vivo* or how they are remodeled throughout the cell cycle. The orientation of the dipole moment of GFP has been well established, and thus, constraining GFP to an endogenous septin allows for an assessment of septin organization *in vivo* by polarization microscopy. Polarized fluorescence analysis has previously shown that septins filaments are paired in properly assembled higher order structures, and organized septin filaments undergo a coordinated 90° reorientation during cytokinesis *in vivo*. We developed a Multifocus Polarization Microscope (MF-PolScope) to evaluate septin organization in 3D through time to capture the assembly and rearrangement of higher-order septin structures, and analysis of mutant yeast strains with abnormally organized septins. MF-PolScope imaging has enabled identification of septin interactors important for distinct aspects of the assembly, stability, and rearrangement of septins. One interactor necessary for proper assembly of septin higher order structure is the regulatory septin Shs1. Using the MF-PolScope we are not only able to show the organization of septin higher order structures, but we are able measure pairing of septin filaments; a measurement that is challenging in the wide-field PolScope system. We were able to identify disorganization of septin structure in Shs1 mutant lines is concomitant with an underlying lack of paired septin filaments. This work has provided more insight into the role of septins in fungal shaping.

167. A trichothecene biosynthetic enzyme complex and a potential mechanism for cellular trichothecene traffic in *Fusarium graminearum*. Karen Broz, Marike Boenisch, Burcu Yordem, H. Corby Kistler. USDA ARS Cereal Disease Lab and University of Minnesota, 1551 Lindig St., St. Paul, MN 55108.

The plant pathogenic fungus *Fusarium graminearum* produces the terpenoid mycotoxin deoxynivalenol (DON) and DON derivatives during infection of wheat as well as when induced in culture. Transcription factor Tri6p regulates expression of genes for enzymes in the mevalonate pathway (primary metabolism) as well as the DON biosynthetic pathway (secondary metabolism). Genes for both pathways display similar patterns of expression during infection, suggesting DON synthesis requires pathway coordination. When strains with fluorescently labeled enzymes are grown in medium conducive to DON induction, the mevalonate pathway enzyme HMG CoA reductase (Hmr1) co-localizes with the DON biosynthetic enzymes Tri1 and Tri4 in spherical membranous structures called "toxisomes". Strict co-localization of Hmr1p, Tri4p, and Tri1p suggest that the enzymes may be part of a multi-enzyme complex, a hypothesis supported by fluorescence resonance energy transfer (FRET) between fluorescently tagged proteins. To further characterize proteins of the toxisome, fluorescence-activated cell sorting (FACS) was used to enrich for Tri4p::RFP tagged toxisomes for proteomic analysis. Preliminary analysis of the FACS-enriched proteome revealed co-enrichment for additional enzymes involved in trichothecene and terpene synthesis. While toxisomes are the proposed site of DON biosynthesis, other subcellular compartments may mediate export of DON. GFP tagging of the trichothecene transporter Tri12p revealed localization to the plasma membrane as well as to vacuoles and small (1 μm) motile vesicles which interact with toxisomes. Motile Tri12 vesicles labeled with GFP fuse with the vacuole or plasma membrane, suggesting that vesicular transport of DON may play a role in cellular sequestration and export of the toxin. The t-SNARE protein directing subapical exocytosis (SSO1) may be involved in toxin export. Deletion mutants (Δsso1) are significantly reduced in the ability to accumulate DON and a DON derivative in toxin induced cultures. Our results suggest a role for vesicular trafficking and exocytosis in export of DON.

168. Protein phosphatase 2A (PP2A) is a subunit of the highly conserved STRIPAK signaling complex controlling fruiting body formation in *Sordaria macrospora*. Anna Beier[1], Dirk Wolters[2], Christoph Krisp[2], Ines Teichert[1], Ulrich Kück[1]. 1) General and Molecular Botany, Ruhr-University Bochum, Germany; 2) Biomolecular Mass Spektrometry, Ruhr-University Bochum, Germany.

The striatin interacting phosphatase and kinase (STRIPAK) complex is involved in diverse developmental processes in eukaryotes and contains at least six highly conserved subunits. In the model ascomycete *S. macrospora*, the STRIPAK complex is involved in hyphal fusion and fruiting body development. From other eukaryotic systems it is known, that S/T protein phosphatase 2A (PP2A) is part of the STRIPAK complex. PP2A balances various signal transduction pathways by regulating kinases or other signaling proteins. This phosphatase is a heterotrimer composed of a structural A-, a regulatory B- and a catalytic C- subunit.

To determine its role for sexual development, we deleted the gene for the catalytic subunit 1 (*pp2Ac1*). The deletion strains are sterile and generate only immature protoperithecia. Catalytic activity of PP2Ac1 was analysed by complementation analysis and *in vitro* activity tests with derivatives of PP2Ac1. In protein-protein interaction studies with PP2Ac1 as bait, we investigated a putative protein supracomplex controlling sexual development in *S. macrospora*. Yeast two-hybrid analysis showed that PP2Ac1 interacts not only with subunits from the STRIPAK complex but also with the PRO40-MAPK interaction network. The PRO40-MAPK interaction network comprises PRO40 and three kinases of the cell wall integrity (CWI) pathway. Cell wall stress tests with STRIPAK mutants showed a functional relationship between STRIPAK and CWI signaling. Phosphorylation levels of Mak1 in STRIPAK mutants underline an association of both protein complexes. Data from tandem affinity purifications with PP2Ac1 as bait followed by mass spectrometry revealed a strong interaction of PP2Ac1 with PRO22 and two a phosphatase associated protein (TAP42). The interaction with TAP42 suggests a link to the target of rapamycin (TOR) pathway. Therefore PP2Ac1 may connect different signaling pathways leading to a controlled reaction on a multitude of signals.

169. *Trichoderma reesei* meiosis generates segmentally aneuploid progeny with higher xylanase-producing capability. Yu-Chien Chuang[1,2], Wan-Chen Li[2,3], Chia-Ling Chen[2], Paul Wei-Che Hsu[2], Shu-Yun Tung[2], Hsiao-Che Kuo[2], Monika Schmoll[4], Ting-Fang Wang[1,2]. 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; 2) Molecular Cell Biology, Taiwan International Graduate Program, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan and Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan; 3) Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan; 4) Austrian Institute of Technology GmbH (AIT), Health and Environment, Bioresources, Tulln, Austria.

Hypocrea jecorina is the sexual form of industrial workhorse fungus *Trichoderma reesei* that secretes cellulases and hemicellulases to degrade lignocellulosic biomass into simple sugars, such as glucose and xylose. The *Hypocrea jecorina* CBS999.97 wild isolate undergoes sexual development to produce fruiting bodies containing asci with 16 linearly arranged ascospores (the sexual spores specific to ascomycetes). By genetic and genomic analyses, we show that the 16 ascospores are generated via meiosis followed by two rounds of postmeiotic mitosis. We also found that the genome of the CBS999.97(1-2) haploid is similar to that of ancestral *T. reesei* strain, whereas the CBS999.97(1-1) haploid genome contains a reciprocal arrangement between two scaffolds of the CBS999.97(1-2) genome. Sexual crossing of these two haploids frequently (>90%) generates asci with viable or inviable ascospores with segmental aneuploidy (SAN). The viable SAN progeny produced white conidia and higher levels of hemicellulases due to segmental deletion and duplication, respectively. Moreover, they readily lose the duplicated segment approximately two weeks after germination. With better lignocellulosic biomass degradation capability, these SAN progeny may provide adaptive advantages to the natural environment, especially in the early phase of colonization.

170. Identification of novel genes regulating sexual development in *Aspergillus* species by functional analysis of transcripts differentially regulated by mating-type loci. Nadhira Salih[1], Adel Ashour[1], Ryuta Wada[2], Junichi Maruyama[2], Katsuhiko Kitamoto[2], Paul Dyer[1]. 1) School of Life Sciences , University of Nottingham, Nottingham NG7 2RD, United Kingdom; 2) Department of Biotechnology, The University of Tokyo, Tokyo 113-8657, Japan.

Sexual morphogenesis in filamentous ascomycete fungi requires the co-ordinated activity of developmental pathways encompassing mating, fruit body production, and meiosis and final generation of ascospores. It has been estimated that at least 400, and probably a much higher number, of genes are needed for normal sexual development. However, a much lower number of genes have so far been identified, indicating that many genes required for sexual morphogenesis remain to be characterised. It is of interest to gain information about such genes as they can provide both fundamental insights into sexual development, and might also provide practical clues as to the genetic basis of asexuality in fungi of applied importance as animal and plant pathogens, and as species used in the biotechnology industry. In the case of *Aspergillus* species approximately 80 genes have so far been described with a proven role in sexual development. Using idiomorph replacement and microarray-based approaches over 30 genes showing greater than 10-fold difference in expression between *MAT1-1* and *MAT1-2* mating types of *A. oryzae* were identified, most of these being of unknown function. We speculated that many of these genes might be required for sexual development. A systematic gene deletion study was therefore undertaken in which candidate genes were deleted from the homothallic (self-fertile) model species *A. nidulans*, and any effect on normal sexual development determined. This led to the discovery of at least 10 novel genes which were required for normal sexual development. Deletion of some genes resulted in total sterility whereas for others a modulation in levels of fertility was evident, suggesting different classes of activity. Gene function was confirmed by complementation by sexual crossing. A model is proposed as to how the newly identified genes relate to those previously involved in sexual development of *Aspergillus* species.

171. The role oxidative stress and RNP granules have in spore survival. Steven Gorsich, Michelle Steidemann, Alyssa Litwiller, Megan Postema. Department of Biology, Central Michigan University, Mt Pleasant, MI.

The meiotic generation of gametes (e.g. spores, pollen grains, oocytes, spermatocytes) is an essential function for sexually reproducing eukaryotes. The ability of plants, animals, and fungi to produce sexual gametes and store them for extended periods of times is essential for the survival of these organisms. For instance, plant pollen grains can be viable for centuries and human oocytes and baleen whales can have viable oocytes for as many as 50 and 90 years, respectively. Interestingly, less is known about the survivorship of fungal spores. Experimental evidence shows that some fungal spores last at least 6 years, while anecdotal evidence suggests that fungal spores can last over 25 years. Though these different types of gametes are less viable as they age, the fact that they remain genetically stable for decades is impressive. Using *Saccharomyces cerevisiae* we are investigating the cellular mechanisms that allow spores to survive for extended periods of time. More specifically, we are interested in the role genetics, environmental stress, and ribonucleoprotein (RNP) granules have on spore development. Previously, we have shown that specific gene mutations (e.g. *dnm1*) lead to defects in spore survival and fitness. In this study, we will present data that describe: 1) how oxidative stress (reactive oxygen species, ROS) effects spore viability and mitochondrial morphology, 2) how the overexpression of *ZWF1* protects yeast against ROS damage during spore development, 3) how the overexpression of *ZWF1* lessens the effects of the *dnm1/dnm1* mutant, and 4) our characterizations of the RNP granules, P-bodies and stress granules, during spore development. These data suggest that *S. cerevisiae* require the ability to protect their developing spores against oxidative stress and the ability to form RNP granules during spore development.

172. The *nsdA4* mutation in NSD204 strain, which is defective in sexual development, is an allele of the *nsdC* gene in *Aspergillus nidulans*. Chae-Ho Lim[1], Mohammed Abdo Elgabbar[1], Dong-Min Han[2], Masayuki Machida[3], Kap-Hoon Han[1]. 1) Dept. Pharmaceutical Engineering, Woosuk Univ, Wanju, Jeonbuk, 565-701, South Korea; 2) 2Division of Life Science, Wonkwang University, Iksan, 570-749, South Korea; 3) 3Bioproduction Research Institute, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Hokkaido 062-8517 Japan.

Fruiting body production and sexual development of fungi are crucial for producing of ascospores by meiosis as well as adapting various environmental changes. In a homothallic fungus *Aspergillus nidulans*, many environmental factors and genes affecting sexual development have been elucidated so far. To investigate sexual developmental process further, NSD mutants, which are defective in the sexual development, have been isolated and characterized. The NSD mutants were divided into four different complementation groups, NSDA-D, and the two genes responsible for the *nsdC* and *nsdD* mutation have already been reported. However, *nsdA4* and *nsdB5* mutations from NSD204 and NSD205 mutants, respectively, are remained to be unveiled. Whole genome sequence of NSD204 mutant obtained from Next Generation Sequencing (NGS) identified possible *nsdA4* mutation candidates. Recent intensive mutation analysis revealed that the NSD204 mutant strain carries missense mutations in *nsdC* ORF region, suggesting that phenotype of NSD204 mutant might be derived from the novel *nsdC* mutation, and eventually indicated that *nsdA4* is an allele of *nsdC* gene. To verify this, NSD204 was genetically crossed with test strain and check the correlation between *nsdA*$^-$ phenotype and *nsdC* mutation. As a result, all strains showing *nsdA*$^-$ phenotype carried *nsdC* mutation which is exactly same mutation found in NSD204 mutant strain, indicating that the *nsdA* gene is identical to the *nsdC* gene.

173. Chromosomal inversion-based mechanism of mating type switching in *Hansenula polymorpha*. Hiromi Maekawa, Yoshinobu Kaneko. Graduate School of Engineering, Osaka University, Suita, Osaka, Japan.

The mating system of Saccharomycotina has evolved from the ancestral heterothallic system as seen in *Yarrowia lipolytica* to homothallism as seen in *Saccharomyces cerevisiae*. The acquisition of silent cassettes was an important step towards homothallism. However, some Saccharomycotina species that diverged from the common ancestor before the acquisition of silent cassettes are also homothallic, including *Hansenula polymorpha*. We investigated the structure of the mating type locus (*MAT*) in *H. polymorpha* and found two *MAT* loci, *MAT1* and *MAT2* that are ~18 kb apart on the same chromosome. The chromosomal location of *MAT1* and *MAT2* was found to influence their transcriptional status, with only one locus maintained in an active state. A histone deacetylase homologous to *S. cerevisiae* Sir2 had no role in this transcriptional repression, suggesting a silencing mechanism distinct from that in *S. cerevisiae*. A

chromosomal inversion of the *MAT* intervening region was induced under mating condition and resulted in the switching of the two *MAT* loci and hence of mating type identity, which was required for homothallism. This chromosomal inversion-based mechanism represents a novel form of mating type switching that requires two *MAT* loci, of which only one is expressed. Furthermore, we investigated the function of the mating type genes. *MAT1*-encoded α1 and *MAT2*-encoded **a**2 specifies α and **a** cell identity respectively, and are required for mating. *MAT1*-encoded α2 and *MAT2*-encoded **a**1 were essential for meiosis. α2 gene was expressed in haploid cells as well as diploid cells. However, splicing of an intron that contains a stop codon occurred only in diploid cells, which may restrict α2 function to meiosis-competent diploid cells.

174. A new STRIPAK interaction partner in *Sordaria macrospora*. Eva Johanna Reschka[1], Oliver Valerius[1], Henning Urlaub[2], Gerhard Braus[1], Stefanie Pöggeler[1]. 1) Genetics of Euk Microorganisms, Georg-August University, Göttingen, Germany; 2) Mass Spectrometry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

The "striatin interacting phosphatase and kinase" (STRIPAK) complex is a conserved multi-protein complex, which is found in mammals, in filamentous fungi and in yeast but not in prokaryotes, and plants[4;5]. Therefore, distinct functions of the STRIPAK complex in different organisms are assumed to depend on the proteins that come together[3]. In *Sordaria macrospora*, the STRIPAK complex proteins SmPP2AA, SmMOB3 and PRO11 are involved in the control of hyphal fusion and fruiting-body development[1;2;6]. PRO11, a homolog of the mammalian striatin, forms homodimers and adopts a propeller-like structure most likely to bring proteins together. The putative kinase activator SmMOB3 contains a conserved MOB domain and further shares homology with a subunit of the clathrin-adaptor protein complex[1]. The scaffolding subunit of the protein phosphatase PP2A (PP2AA) interacts with regulatory B subunits that associate with the STRIPAK complex for the recruitment of substrates and the targeting to subcellular compartments[4]. GFP-Trap and mass spectrometry were used to identify new STRIPAK complex components in *S. macrospora*. Known interaction partners of PRO11 such as SmMOB3 and PRO22 could be verified. Interestingly, a protein with unknown function, which we termed SCI1 (STRIPAK complex interactor 1) was identified. SCI1 seems to be conserved in fungi and was predicted to contain coiled-coil regions. We assume that SCI1 may have the same structural role in the STRIPAK complex of *S. macrospora* like FGFR1OP2 and SIKE in humans[4]. Here, we present data of the functional analysis of the protein SCI1 and the characterization of the interaction of SCI1 with STRIPAK components in *S. macrospora*.

1 Bernhards and Pöggeler, Curr Genet 2011; 57:133-49. **2** Bloemendal et al., Mol Microbiol 2012; 84:310-23. **3** Frost et al., Cell 2012; 149:1339-52. **4** Goudreault et al., Mol Cell Proteomics 2009;8:157-71. **5** Kemp and Sprague, Mol Cell Biol 2003; 23:1750-63. **6** Pöggeler and Kück, Eukaryot Cell 2004; 3:232-40.

175. Regulation of sporulation in *Ashbya gossypii*. Lisa Wasserstrom, Klaus Lengeler, Andrea Walther, Jurgen Wendland. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Denmark.

Sporulation is a tightly regulated process and in *Ashbya gossypii* endospores are formed by a *MATa* mycelium upon nutrient starvation. We have shown previously that mating prior to endospore formation is not a prerequisite in *A. gossypii*. Additionally, we identified three genes, *STE11*, *STE7* and *STE12*, which result in a hypersporulation phenotype when deleted.

We now have expanded our analysis on transcriptional regulation upstream of *IME1*. Ime1 is a central regulator of sporulation in *S. cerevisiae*, and its homolog in *A. gossypii* is essential for sporulation as well. Here we elucidate the role of the Ras/cAMP-dependent protein kinase A (PKA) pathway in regulating the switch between vegetative growth and sporulation in *Ashbya gossypii*.

Sporulation is completely blocked by exogenous cAMP in the wild type strain and the *tpk1* mutant but not in *tpk2*. Tpk1 is, therefore, not sufficient to compensate for the loss of Tpk2 and to block sporulation. Functional divergence between Tpk1 and Tpk2 also extends to spore germination. Spores of *tpk2* strains, but not *tpk1* strains, are severely deficient in breaking the dormancy of spores and germ cell formation.

Downstream targets of PKA are *MSN2* and *SOK2* in *S. cerevisiae*. Loss of *SOK2* in *Ashbya* abolishes sporulation and riboflavin production, while deletion of *MSN2* shows reduced sporulation indicating that Sok2 and Msn2 act as positive sporulation regulators. In contrast, Sok2 in *S. cerevisiae* is a repressor of sporulation.

All strains were analysed by RNAseq, which allowed us to identify the set of genes upregulated in sporulation. Together with our previous findings we propose a specific evolutionary adaptation of *A. gossypii* to its biological niche allowing sporulation in the absence of mating and promoting fast sporulation.

176. The selective autophagy cargo receptor SmNBR1 in the ascomycete *Sordaria macrospora*. Antonia Werner, Oliver Valerius, Gerhard Braus, Stefanie Pöggeler. Genetics of Euk Microorganisms, Georg-August University, Göttingen, Germany.

Autophagy is a conserved ubiquitous degradation process in eukaryotic cells. It includes the random sequestration of defective and excessive proteins and organelles within a double-membraned autophagosome [1]. Typically, it is induced by starvation or stress conditions. After induction the autophagosomal membrane engulfs cytosolic cell contents and delivers it to the vacuole for degradation. Besides this non-selective autophagy processes, selective autophagy of damaged or harmful protein aggregates and surplus organelles such as peroxisomes, mitochondria, ribosomes and nuclei is referred to as aggre-, pexo-, mito-, ribo-, and nucleophagy, respectively. In filamentous fungi, it has been investigated that autophagy is involved in vegetative development and pathogenicity [2]. *Sordaria macrospora* is a filamentous ascomycete and many genes of the autophagic system are conserved [2,3]. So far, cargo receptors for selective autophagy have not been described in filamentous fungi. In all eukaryotes, the autophagy related (ATG) protein ATG8, is essential for the autophagosomal

membrane formation during expansion. ATG8 is conjugated to phosphatidyl-ethanolamine and localizes to the autophagosomal membrane [4]. Interaction partners of SmATG8 were identified by GFP-Trap and LC-MS analysis [3]. One of the interacting proteins, SmNBR1, is a putative homolog of the human NBR1 (neighbor of BRCA1 gene1) and harbors an ATG8 interacting region called LIR (LC3-interacting region). In human NBR1, has been described as a receptor for selective autophagy. It targets ubiquitinylated protein aggregates to autophagosomes and is involved in neurodegenerative diseases and cancer [5]. The interaction of SmATG8 and SmNBR1 was confirmed by means of yeast two-hybrid experiments and fluorescence microscopy. A ΔSmnbr1 mutant could be generated which is impaired in vegetative growth and fruiting-body development. 1) Reggiori and Klionsky (2007) Autophagy 3(5):502-505; 2) Voigt and Pöggeler (2013) Appl Microbiol Biotechnol 97:9277-9290; 3) Voigt and Pöggeler (2013) Autophagy 9:1-17; 4) Geng and Klionsky (2008) Embo Rep 9:859-864; 5) Kirkin et al (2009) Mol Cell 33:505-516.

177. Unisexual reproduction in *Huntiella moniliformis*. A.M. Wilson[1], T. Godlonton[1], M.A. van der Nest[1], P.M. Wilken[1], M.J. Wingfield[2], B.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.

Sexual reproduction in fungi is controlled by genes present at the mating type (*MAT*) locus, which typically harbours transcription factors that influence the expression of many sex-related genes. The *MAT* locus exists as two alternative idiomorphs in ascomycetous fungi and sexual reproduction is initiated when genes from both idiomorphs are expressed. Thus, the gene content of this locus determines whether a fungus is heterothallic (self-sterile) or homothallic (self-fertile). Recently, a unique sub-class of homothallism has been described in fungi, where individuals possessing a single *MAT* idiomorph can reproduce sexually in the absence of a partner. Using various mycological, molecular and bioinformatic techniques, we investigated the sexual strategies and characterized the *MAT* loci in two tree wound-infecting fungi, *Huntiella moniliformis* and *H. omanensis*. *H. omanensis* was shown to exhibit a typically heterothallic sexual reproductive cycle, with isolates possessing either the *MAT1-1* or *MAT1-2* idiomorph. This was in contrast to the homothallism via unisexual reproduction that was shown in *H. moniliformis*, where only the *MAT1-2-1* gene was present in sexually reproducing cultures. While the evolutionary benefit and mechanisms underpinning a unisexual mating strategy remain unknown, it could have evolved to minimize the costs, while retaining the benefits, of normal sexual reproduction.

178. Molecular analysis of unidirectional mating type switching in *Chromocrea spinulosa*. Sung-Hwan Yun[1], Hee-Kyoung Kim[1], Theresa Lee[2], B. Gillian Turgeon[3]. 1) Dept Medical Biotechnology, Soonchunhyang Univ, Asan, Chungnam, South Korea; 2) Microbial Safety Team, NAAS, RDA, Wanju, South Korea; 3) Plant Pathology & Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell Univ, Ithaca, NY.

For most filamentous ascomycetes, a single mating type (*MAT*) locus with two alternate idiomorphs (*MAT1-1* and *MAT1-2*) controls mating ability. Heterothallic species carry these alternate forms in different nuclei, and require a partner of opposite mating type to mate, whereas most homothallic species carry both *MAT1-1* and *MAT1-2* in a single nucleus and can self mate. *Chromocrea spinulosa* (*Hypocrea spinulosa*) undergoes unidirectional switching from a homothallic to heterothallic mating strategy. Sequencing of *MAT* regions reveal that both homothallic and heterothallic strains carry three *MAT1-1* genes (*MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3*), while self-fertile strains carry an additional, linked, *MAT1-2-1* gene. The latter is flanked by a 260-bp repeat which is present as a single copy at *MAT* of self-sterile strains. Molecular genetic manipulations suggest that: i) the presence of both *MAT* idiomorphs in a single nucleus is not sufficient for self-fertility, ii) self-fertility likely results from a recognition event between a nucleus carrying both mating types and a nucleus carrying only *MAT1-1*, iii) looping out of *MAT1-2-1 via* homologous recombination between the 260-bp repeats is a likely mechanism for unidirectional switching.

179. Ethylene response of the plant-fungal fusion histidine kinase in yeast and filamentous fungi. Mayumi Nakayama[1,2], Kentaro Furukawa[3], Akira Yoshimi[2], Keietsu Abe[1,2]. 1) Dept Microbial Biotechnology, Grad. Sch. Agricult. Sci., Tohoku Univ., Sendai, Miyagi, Japan; 2) New Industry Hatchery Center, Tohoku University, Sendai, Miyagi, Japan; 3) University of Gothenburg, Gothenburg, Sweden.

The two-component signal transduction system (TCS) has been conserved widely in bacteria and eukaryotes, including plants and fungi. TCS typically consists of two types of common signal transducers: histidine kinase (HK), a response regulator (RR). In the plant *Arabidopsis thaliana*, the ethylene receptor AtETR1 acts as HK and its HK activity is regulated by ethylene. AtETR1 contains (i) an ethylene-binding domain (EBD) consisting of three transmembrane helices in the N-terminal half, (ii) an HK domain (HKD) containing HK, and (iii) a receiver domain of RR in the C-terminal half. As well as AtETR1, fungal HKs also consist of an N-terminal sensor detecting environmental stimuli, an HKD and an RR. Fungal signal transduction pathways containing these fungal sensors control cellular responses to extrinsic and intrinsic signals. If these fungal sensor domains are replaced by EBD of AtETR1 and fungal signal pathways (e.g. HOG-pathway) can be controlled by ethylene, the hybrid HKs would be useful as a new gene regulation system in fungal industry. To create a novel system of gene regulation by ethylene, we constructed and examined expression systems of plant-fungal fusion HKs in yeast and filamentous fungi. Then we confirmed functional complementation and ethylene response of plant-fungal fusion HKs in a temperature-sensitive *sln1* yeast mutant and *Aspergillus nidulans*. Here, we report experiments for optimization of ethylene response using HOG-dependent reporter systems, *8xCRE-lacZ* and their modified genes.

180. The conserved and divergent roles of the MAP kinase gene, *mpkB*, in *Aspergillus flavus*. Sang-Cheol Jun[1], Kwang-Yeop Jahng[1], Dong-Min Han[2], Kap-Hoon Han[3], Jong-Hwa Kim[3]. 1) Life Science, Chonbuk National University, Jeonju, Jeonbuk, South Korea; 2) Division of Life Science, Wonkwang University, Iksan, 570-749, South Korea; 3) Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, South Korea.

Eukaryotes' developmental processes are controlled by the multiple regulatory systems. Among them, MAP kinase pathways play important roles in regulation of growth, development, and stress responses. To characterize the function of MAP kinase in an important pathogenic and toxigenic fungus *Aspergillus flavus*, the *AflmpkB* gene (AFL2G_02589), an orthologue of the yeast *FUS3* gene and the *mpkB* gene of *Aspergillus nidulans*, was deleted and analyzed. In *A. nidulans*, previous studies revealed that MpkB positively regulates the

sexual and asexual differentiation as well as secondary metabolite production. In this study, deletion of *AflmpkB* resulted in no mycelial growth change, while the conidial production was reduced about 60% comparing to the wild-type. Also, the mutant produced immature and abnormal conidiophores such as vesicular dome-immaturity in the conidiophore head, decreased number of the phialides and very short stalks, although expression of the *brlA* gene, a key regulator of conidiation, was up-regulated in the mutant. Moreover, Δ*AflmpkB* couldn't produce any sclerotia, suggesting that the *AflmpkB* gene plays an important role not only in conidiophore generation but also in sclerotia development. However, unlike *A. nidulans* where the sterigmatocystin biosynthesis was strongly decreased in *mpkB* mutant, *AflmpkB* mutants produced normal level of aflatoxin B_1. Taking together, *A. flavus mpkB* gene plays a positive regulatory role in the production of the conidiation and the sclerotia formation but not in the production of the secondary metabolites including aflatoxin B_1.

181. *Phytophthora infestans* GPCR-PIPK GK4: a membrane localized receptor with PI4P 5-kinase activity. Johan van den Hoogen, Chenlei Hua, Harold Meijer, Francine Govers. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Signaling networks involving heterotrimeric G-proteins and phospholipids are fundamental to many cellular processes in eukaryotes. Oomycetes possess a family of twelve novel proteins called GPCR-PIPKs (GKs) that are composed of a N-terminal G-protein-coupled-receptor (GPCR) domain fused to a phosphatidylinositol phosphate kinase (PIPK) domain. Based on this structure GKs are anticipated to link G-protein and phospholipid signaling but their functions and biochemical activities are unknown. Previously we analyzed the function of PiGK4 in the potato late blight pathogen *Phytophthora infestans* by gene silencing and overexpression and showed its involved in spore development, sporangial cleavage, hyphal elongation and virulence (Hua, Meijer et al. 2013, Mol. Microbiol.). Microscopic analysis revealed that fluorescently tagged full length and truncated versions of PiGK4 localize to membranes surrounding certain cellular compartments. Since GKs are involved in developmental transitions in *Phytophthora* and GPCRs are major drug targets, GKs have potential as oomicide targets. To determine the enzymatic activity of PiGK4 we exploit the temperature sensitive yeast mutant *mss4*^{ts} that lacks PI4P 5-kinase activity when grown at the restrictive temperature. Complementation is achieved with full-length PiGK4 but not with a truncated PiGK4 without GPCR domain. Apparently PiGK4 has the same enzymatic activity as yeast Mss4p but requires the GPCR domain to function, possibly by targeting the PIPK to its substrate which is likely a membrane associated phosphoinositide. Here we present a more detailed analysis of the function of the various conserved domains in PiGK4 by complementation assays in *mss4*^{ts} using modified versions of PiGK4 generated by deleting and swapping domains.

182. The inositol polyphosphate phosphatase family in oomycetes. Johan van den Hoogen, Kelly Heckman, Charikleia Schoina, Francine Govers, Harold Meijer. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Phosphoinositides (PIs) play essential roles in intracellular transport and communication. They function as membrane signalling molecules and modulate the activity of a plethora of proteins. Eukaryotic cells harbour seven distinct PI isoforms, the levels of which are governed by the activity of PI kinases (PIKs and PIPKs) and inositol polyphosphate phosphatases (INPPs) that rapidly convert one PI into another. Previously we identified a unique repertoire of PI(P)Ks in *Phytophthora* spp. including twelve GPCR-PIPKs (GKs) composed of a N-terminal G-protein-coupled-receptor (GPCR) domain fused to a PIPK catalytic domain. GK4 and GK5 have been shown to be involved in developmental transitions in *Phytophthora* (Yang et al. 2013, Mol. Microbiol.; Hua et al. 2013, Mol. Microbiol.). Here we focus on *Phytophthora* INPP genes. A genome-wide inventory of *Phytophthora* spp. and other oomycetes revealed that most genomes encode around 22 distinct INPPs. They are well conserved in their catalytic domains and correlate to the known INPPs classes (INPP3, -4, -5 and SAC-like lipid phosphatases). However, in a subset of the INPPs the catalytic region, either INPP4 or INPP5 is fused to an N-terminal GPCR moiety. These GPCR-INPPs (GIs) thus resemble the GKs and this structural similarity points to a role in spatiotemporal distribution of PIs at distinct membranes. With the ongoing research on putative phospholipid-based transport of effectors that are essential for pathogenicity it is worth to investigate the *in vivo* functions of GIs. Moreover, since GPCRs are the main drug targets, GKs and GIs might have potential as oomicide targets.

183. A regulator of G protein signaling connecting sexual development with the volatilome of *Schizophyllum commune*. Sophia Wirth[1], Katrin Krause[1], Maritta Kunert[2], Wilhelm Boland[2], Erika Kothe[1]. 1) Microbial Communication, Friedrich Schiller University, Jena, Germany; 2) Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany.

Fungi are able to produce a wide range of volatile organic compounds, including hydrocarbons, alcohols, ketones, ethers, esters and terpenes. The chemical composition of volatiles emitted by the basidiomycete *Schizophyllum commune* was investigated using solid phase microextraction coupled with GC-MS. In addition, the volatile spectra of mutant strains containing a transposon insertion in a regulator of G protein signaling gene (*thn*) were analyzed. Thn negatively regulates pheromone receptors and thereby prevents pheromone signaling. This is reflected in the volatilome. The wild-type was found to produce mainly esters, whereas *thn* mutant strains were found to produce a mixture of different sesquiterpenes, including α- and β-bisabolol. Sesquiterpenes have diverse ecological functions, e.g. as autoinducers, in attraction of pollinators or as defense compounds. In bioassays, volatiles of *S. commune thn* mutants inhibit the growth of *Pleurotus ostreatus* and *Flammulina velutipes*, while their hyphal morphology remains unaffected. It could be shown that the sesquiterpenes α-bisabolol and bisabolene contribute to the observed growth inhibition. In the genome of *S. commune*, genes required for sesquiterpene production *via* the mevalonate pathway have been identified. To link regulation to modification, a proteome analysis was performed. Differentially expressed proteins belonged to the functional classes of cellular processing and signaling as well as metabolic processes. Transcriptome analysis revealed a regulation of genes involved in the synthesis of sesquiterpenes and indicates a genetic connection between pheromone signaling and secondary metabolism. To investigate the molecular mechanism regulating volatile production, the deletion of *thn* will be performed.

184. Mechanical stress initiates intercalary growth in *Epichloë* fungal symbionts of grasses. K. G. Sameera 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) Plant Fungal Interactions, AgResearch Grasslands, Palmerston North, Manawatu, New Zealand; 2) Institute of Fundamental Studies, Massey University, Palmerston North, New Zealand; 3) School of Medical Sciences, University of Aberdeen, United Kingdom; 4) Manchester Fungal Infection Group, University of Manchester, United Kingdom; 5) AgResearch, Lincoln Research Centre, Christchurch, New Zealand.

Colonization of aerial grass tissues by seed-transmitted *Epichloë* endo-symbionts initially occurs through ramification of hyphal tips between cells of the host shoot apical meristem (SAM). Uniquely, when hyphae in the SAM start to invade developing leaves, growth ceases at apices, and hyphae extend via intercalary growth (division and extension in non-apical compartments). We hypothesise that intercalary growth is stimulated by mechanical stretch imposed on hyphae by their attachment to elongating host cells, and that this stress is sensed by mechano-sensors located on hyphal membranes. Deletion of *E. festucae mid1*, a putative orthologue of the *mid1* yeast mechano-sensor, and a component of the Mid1/Cch1 calcium channel, reduced *E. festucae* radial growth rate in culture, caused aberrations in hyphal cell walls, and greatly restricted intercalary growth in infected plants. A technique to mimic the hyphal stretching proposed to occur *in planta* has been developed and tested on wild type *E. festucae* growing in culture. Intercalary compartments remained viable despite being stretched to 20% of their original length, and stretching also initiated *de novo* mitosis and septation in intercalary compartments. Calcium imaging experiments on *E. festucae* growing in culture have revealed that the Mid1 protein is responsible for calcium pulses at the hyphal tip during growth, and that the calcium originates from the exterior of the hypha and not from calcium stores. Studies are underway to characterise calcium signalling in intercalary compartments in wild type and the Δ*mid1* deletion mutant when subjected to mechanical stress.

185. Sexual development in basidiomycetes – on the role of additional receptor genes. Erika Kothe, Daniela Freihorst, Elke-Martina Jung. Dept Microbiol, Microbial Communication, Friedrich Schiller University, Jena, Germany.

The tetrapolar mating system within the basidiomycetes has proven to be ever more complex. Within the B mating type loci, multispecific, multiallelic pheromone and receptor containing subloci contribute to the more than 23,000 mating types occurring, e.g., with Schizophyllum commune. A striking novelty to the system is the detection of several non-mating receptor (NMR) genes, some of which are present within and others outside the mating type loci. Here, the potential function of such NMRs, their regulation during diffent stages of sexual development and hyphal growth, and hypotheses for involvement in the complex system of recognizing mates is discussed.

B-regulated signaling in sexual development can be re-assessed making use of transcriptome data obtained with several mutant strains in pheromone signalling, Ras signalling and regulation of pheromone receptor gene expression. Evolutionary aspects can be addressed from synthetic construction of new mating types not present in nature.

Analysis of other basidiomycete genome sequences is used to discuss the distribution and evolution of both, the complex mating system and the presence and potential function of NMRs.

186. Pex5-dependent Peroxisomal Import in *Ustilago maydis*. Julia Ast, Domenica Martorana, Johannes Freitag, Michael Bölker. Biology, Philipps University Marburg, Marburg, Germany.

Peroxisomes are ubiquitous organelles that perform important metabolic reactions such as the β-oxidation pathway for degradation of fatty acids. Peroxisomal proteins are translated in the cytosol and are imported as fully folded and co-factor bound proteins and even as oligomers. The vast majority of peroxisomal proteins contain a short conserved C-terminal targeting signal (PTS1). These proteins are imported by a conserved cytosolic receptor protein, Pex5. Only a few peroxisomal proteins carry an N-terminal targeting signal (PTS2), which is recognized by the receptor protein Pex7.

The plant pathogenic fungus *Ustilago maydis* encodes two Pex5 receptors (Pex5a, Pex5b), which show a high extent of sequence similarity. Deletion of *pex5a* abolished growth on oleic acid but has nearly no effect on pathogenic development. In contrast, Pex5b was found to be important not only for growth on fatty acids, but also for filament formation and virulence. We could show that both Pex5a and Pex7 depend on the presence of Pex5b for import. Therefore, we assume that Pex5b serves as co-receptor for both Pex5a and Pex7. Transcription of *pex5a* and *pex5b* is differentially regulated during the life cycle of *U. maydis* suggesting modulation of the peroxisomal proteome and metabolism during infection..

Comparative and Functional Genomics

187. Identification and Characterization of Histone Genes in the *Coprinopsis cinerea* Genome. Marilee Ramesh, Kelsey Greene, Alexandra Warn. Department of Biology, Roanoke College, Salem, VA.

Histones, highly conserved proteins found in all eukaryotes, are fundamental for packaging and organizing DNA within the nucleus. Canonical histones and histone variants have been identified and characterized in the *Coprinopsis cinerea* genome. Bioinformatics techniques were used to identify these genes based on unique structural characteristics and sequence similarity in closely related organisms. Histones were further classified by determining copy number and location in the genome. Evidence for expression was determined based on reviewing genome-wide EST and SAGE analyses. This study resulted in the identification of twelve core histones: three H2As, two H2Bs, three H3s and three H4s. These genes were distributed in two clusters on chromosomes seven and nine. Three H1 linker histones have been identified in a cluster on chromosome two. The pattern of clustering is highly similar to that observed in both closely related basidiomycete species and higher organisms.

188. Simple Synteny: An accessible tool for genome comparison. D. Veltri[1,2,3], M. Malapi-Wight[1], J.A. Crouch[1]. 1) Systematic Mycology & Microbiology Laboratory, USDA-ARS, Beltsville, MD USA; 2) Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ USA; 3) School of Systems Biology, George Mason University, Manassas, VA USA.

Understanding the co-localization of genetic loci amongst species is a frequent task of many biologists. While there are a number of programs currently available for generating syntenic maps, many are inaccessible to biologists who lack the required computer or scripting skills needed for proper installation or data preprocessing. We present here Simple Synteny, a free and open-source image generator for use in syntenic analysis. Simple Synteny provides an easy interface for comparing multiple genomes by only requiring the user to submit FASTA files for the genomes and genes of interest. The program employs gene color coordination across genomes, indicator arrows for changes in gene direction and automatic contig and gene resizing to produce publication-quality images which are clear and simple to interpret. Directions for using the tool and example analyses are provided.

189. Whole genome sequencing of the blue mold fungus, *Penicillium expansum*. Jiujiang Yu[1], Wayne Jurick[1], Yanbin Yin[2], Verneta Gaskins[1], Liliana Losada[3], William Nierman[3], Joan Bennett[4]. 1) USDA/ARS, Beltsville Agricultural Research Center, Beltsville, MD. 20705; 2) Department of Biological Sciences, Northern Illinois University, Dekalb, IL 60155; 3) The J. Craig Venter Institute, Rockville, MD 20850; 4) Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901.

P. expansum is the most virulent and economically significant species that causes blue mold of apple and pear fruit during storage and produces mycotoxins (i.e. patulin) that impact human health. To understand the genetic mechanism(s) controlling virulence and other intriguing biological processes in this fungus, the whole genome sequence of a wild-type *P. expansum* isolate was obtained using Next Generation Sequencing Technology. The sequences were assembled and annotated. The resulting *P. expansum* genome was estimated to be 31,415,732 bp in size which is congruent with what has been reported for *P. chrysogenum.* The fungus has 10,554 predicted genes, with an average length of 1,599 bp. The total coding sequence is 16,873,185 bp, which makes up 53.70% of the fungal genome. The draft *P. expansum* genome sequence serves as a platform to functionally analyze specific genes involved in pathogen virulence to design much needed control strategies to combat blue mold decay during storage.

190. The *Aspergillus fumigatus* genome-wide knock out library. Juliane Macheleidt[1], Thorsten Heinekamp[1], Vito Valiante[1], Fabian Horn[2], Reinhard Guthke[2], Paul Carr[3], Jane Gilsenan[3], Isabelle Mouyna[4], Jean-Paul Latgé[4], Michael Bromley[3], Axel Brakhage[1]. 1) Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Molecular and Applied Microbiology, Jena, Germany; 2) Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Systems Biology and Bioinformatics, Jena, Germany; 3) Institute of Inflammation and Repair, University of Manchester, UK; 4) Institute Pasteur, Aspergillus Unit, Paris, France.

The opportunistic human pathogenic mould *Aspergillus fumigatus* is able to cause severe invasive infections. The most powerful tool to study the role of certain genes and proteins in virulence of *A. fumigatus* is to generate gene knock outs and to analyze the loss of function mutants. Up to now, these deletion strains were generated separately for each gene of interest, which is often a time consuming process. To facilitate and accelerate research on *A. fumigatus*, we started to build up a library of deletion mutants for all of the approximately 10,000 genes of this fungus.

Prerequisite for a knock out library are accurately annotated genes. Therefore, the genome of the *A. fumigatus* A1163 derivative Δ*akuB* was re-sequenced and RNAseq data were used for re-annotation. Based on that, gene deletion primers were generated in an automated process and a streamlined workflow combining generation of deletion cassettes by a two-step PCR reaction, protoplast-based transformation, isolation of genomic DNA and verification of the successful deletion by PCR was developed in 96-well plate format for high throughput application. Based on the introduction of an MmeI restriction site to the deletion cassette, each mutant carries a unique barcode allowing identification of specific strains when pools of mutants are tested. As a first subset, we deleted 132 glycosylphosphatidylinositol (GPI) anchored protein encoding genes and started phenotypic analysis of the resulting mutant strains.

191. Attenuation of gliotoxin self-protection in *A. fumigatus* reveals global effects on the cell, in particular on secondary metabolism. Grainne O' Keeffe[1], Stephen Hammel[1], Rebecca Owens[1], Thomas Keane[2], David Fitzpatrick[1], Gary Jones[1], Sean Doyle[1]. 1) Department of Biology, Maynooth University, Co. Kildare, Ireland; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, United Kingdom.

Aspergillus fumigatus produces a number of secondary metabolites, one of which, gliotoxin, has been shown to exhibit anti-fungal activity and consequently an endogenous self-protection system is required. One of the genes in the gliotoxin biosynthetic gene cluster in *A. fumigatus*, *gliT*, is required for self-protection against the toxin- however the global self-protection mechanism deployed is unclear. RNA-seq identified genes differentially regulated upon exposure to gliotoxin in *A. fumigatus* wild-type and *A. fumigatus ΔgliT*, a gliotoxin hypersensitive strain. Expression of 164 genes was differentially regulated ($\log_2$ fold change of 1.5) in *A. fumigatus* wild-type when exposed to gliotoxin, consisting of 101 genes with up-regulated expression and 63 genes with down-regulated expression. Interestingly, a much larger number of genes, 1700, were differentially regulated ($\log_2$ fold change of 1.5) in *A. fumigatus ΔgliT* when challenged with gliotoxin. These consisted of 508 genes with up-regulated expression, and 1192 genes with down-regulated expression. FunCat classification of differentially regulated genes revealed an enrichment of genes involved in both primary metabolic functions and secondary metabolism. Specifically, genes involved in gliotoxin biosynthesis, helvolic acid biosynthesis, siderophore-iron transport genes and also ribosome biogenesis genes underwent altered expression. It was confirmed that gliotoxin biosynthesis is induced upon exposure to exogenous gliotoxin, production of unrelated secondary metabolites is attenuated in *A. fumigatus ΔgliT*, while quantitative proteomic analysis confirmed disrupted translation in *A. fumigatus ΔgliT* challenged with exogenous gliotoxin. Our data highlight the global and extensive affects of exogenous gliotoxin on a sensitive strain devoid of a self-protection mechanism and infer that GliT functionality is required for the optimal biosynthesis of selected secondary metabolites in *A. fumigatus*.

192. Bulk segregant analysis followed by high-throughput sequencing identifies a novel gene required for acid production in Aspergillus niger. Jing Niu[1], Peter J. Punt[1,2], Arthur F. J. Ram[1]. 1) Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands; 2) TNO Microbiology and Systems Biology, PO Box 360, 3700 AJ Zeist, The Netherlands.

We have combined high-throughput sequencing (Illumina) with bulk segregant analysis to identify the mutation in a previously isolated non-acidifying mutant in Aspergillus niger. Because of the lack of a sexual cycle for A. niger, the parasexual cycle was used to generate a pool of segregants. A set of defined color mutants with auxotrofic markers was constructed by targeted gene deletion to facilitate the construction of diploid strains in A. niger. In the bulk-segregant analysis approach, the mutant of interest is crossed to a wild-type strain and haploid segregants displaying the phenotype of interest are pooled and DNA from this pool of segregants is sequenced using a deep sequencing technology (e.g. Illumina). The mutation causing the non-acidifying phenotype was found to be recessive and since about 50% of the segregants (78 of the 140 segregants analysed) showed the non-acidifying phenotype, the phenotype was likely to be caused by a single mutation. In addition to sequencing the genomic DNA pool of segregants, the parental strains were also sequenced and single nucleotide polymorphisms (SNPs) between the strains were identified. SNPs between the parental strains not related with the phenotype, have a 50% chance to be present in the pool; SNPs responsible for the phenotype or closely linked to the mutation responsible for the phenotype, are conserved in the pool. In total, 52 SNPs were identified between the two parental strains and three SNPs were 100% conserved in the pool of segregants. All three SNPs mapped to the right arm of Chromosome II, indicating that this region contains the genetic locus affecting the phenotype related to acid production. It is currently determined which of the three SNP is responsible for the non-acidifying phenotype of the mutant by complementation and targeted deletion studies.

193. *Aspergillus niger* regulatory mutant strains for improved protein production: strain selection and mutant gene identification. Peter Punt[1], Jing Niu[2], Deepa Nair[1,2], Ellen Lagendijk[2], Mark Arendhorst[2], Arthur Ram[2]. 1) Microbiology, TNO, Zeist, Netherlands; 2) Leiden University, Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands.

Optimized protein production in filamentous fungi requires the availability of fungal strains with low levels of secreted protease activity. Already for several decades research has been carried out to obtain these type of mutants, leading to the isolation of mutants with very favourable characteristics. Complementation studies have allowed identification of several of these mutants, one being a mutation in a transcriptional regulatory gene, prtT (e.g. Punt et al;., 2008).

Based on this mutant strain further improved strains have been selected using positive selection approaches. Controlled fermentation experiments with these strains revealed different protease profiles, whereas full genome sequencing was carried out in an attempt to identify the genetic basis of the mutant phenotypes.

Another method to identify regulatory protease deficient mutants, was based on the use of collections of regulatory gene knock-out strains in N. crassa and A. niger. Based on positive selection approaches and classical milkhalo screening novel mutant strains with modified protease production profiles were obtained.

194. Comparative genomics and gene cluster identification in 28 species of *Aspergillus* section *Nigri*. Tammi Vesth[1], Jane Nybo[1], Sebastian Theobald[1], Ellen K. Lyhne[1], Martin E. Kogle[1], Igor Grigoriev[3], Uffe H. Mortensen[1], Scott E. Baker[2], Mikael R. Andersen[1]. 1) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Joint BioEnergy Institute, Emeryville, CA and Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA; 3) Joint Genome Institute, Walnut Creek, CA, USA.

The filamentous fungus *Aspergillus niger* and its close relatives in *Aspergillus* section *Nigri* are of broad interest to the scientific community including applied, medical and basic research. The fungi are prolific producers of native and heterologous proteins, organic acids (in particular citrate), and secondary metabolites (including bioactives and toxins such as ochratoxin A). Because of these abilities they represent a substantial economic interests in bioenergy applications. While 8 individual species from this group has been whole-genome sequenced, the genetic basis for these diverse phenotypes remains largely unidentified.
In this study, we have de novo sequenced the genomes of 20 additional species of the section *Nigri*, thus allowing the genome comparison of all members of this important section of fungal species. Here we present the results of this large-scale genomic analysis where we have examined the core genome of these 28 species and identified variations in the genetic makeup of individual species and groups of species. In particular, we have found genes unique to *Aspergillus* section *Nigri*, as well as genes which are only found in subgroups of the section. Our analysis here correlates these genes to the phenotypes of the fungi.
Furthermore, we have predicted secondary metabolite gene clusters in all 28 species. We present here an overview of these gene clusters and how they are shared and vary between species. We also correlate the presence of gene clusters to presence of known fungal metabolites.

195. Comparison of a commercial *Aspergillus oryzae* strain and its degenerated strain by genome re-sequencing. Yiyi Zhong, Wenyan Nong, Hoishan Kwan. CUHK, Shatin, NT, HK.

The filamentous fungus *Aspergillus oryzae* plays a critical role in the koji-making process for soy sauce fermentation. It can produces many enzymes to extensively digest macronutrients which gives soy sauce a pleasant combination of flavor and appearance. The strain RD2 is commercially used in Chinese soy sauce industry. A degenerated strain TS2 obtained during production would yield poor-quality soy sauce when used in koji making. We aimed to explain the phenotypic differences of two strains at the molecular level by genomic

comparison. We first applied Ion-torrent technology to sequence RD2 and TS2 genomes. Three loci, beta tubulin, calmodulin and RNA polymerase II, from 21 *Aspergillus* strains were used for genealogical concordance analysis. From the phylograms, industrially important strains form a strongly supportive clade. TS2 is a closest relative of RD2, which is also closely related to the Japanese strain RIB40 and the Chinese strain 3.042. Then we mapped RD2 and TS2 sequences separately to the RIB40 genome. There are 7721 SNPs and 3463 deletions in annotated genes. Among them, those related to the genes of hydrolytic process, including amylases, proteases, nucleotidases and lipases, were selected for comparison. The analysis reveals that TS2 has unique SNPs in many genes including alpha-amylase, cysteine protease atg4 and phospholipase D precursor. Unique deletions of TS2 occurred in several proteases related to amino acid metabolism, regulation and signaling, in 2 nucleotidases namely IMP-specific nucleotidase, biphosphate nucleotidase and several lipases. Though no evidence of a single gene loss, these unique genetic variations are likely to cause disruption of gene function in macronutrients digestion and flavor formation. Based on the genomic comparison, we can attribute the enzyme production differences between RD2 and TS2 to their genetic variation. Further, we infer that the genomic variations in TS2 affect the secretory enzymes and soy sauce production using the strain. More significantly, this study provides a genomic foundation for transcriptomic and proteomic study of the commercial strains. In the long term, we can resolve the mechanism of hydrolytic digestion in soy sauce production.

196. Comparative transcriptome analysis of plant biomass degradation by filamentous fungi. Magdalena A. Kolbusz, Nadeeza Ishmael, Justin Powlowski, Adrian Tsang. Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada.

Plant biomass is the most abundant carbon source in the biosphere and can be efficiently utilized by fungi using various extracellular carbohydrate active enzymes (CAZymes). Here we have examined by transcriptome analysis how phylogenetically distant fungi utilize two types of complex biomass, i.e. barley (monocot) and alfalfa (dicot) straws. The major components in barley straw are cellulose and xylan while alfalfa straw is composed mainly of cellulose, xylan and pectin. We focused on the expression of genes encoding cellulases, xylanases, pectinases and mannanases for this presentation. Five fungal species were compared: *Trametes versicolor* representing Basidiomycota, *Rhizomucor pusillus* representing Mucoromycotina, as well as *Mycothermus thermophilus*, *Myceliophthora thermophila* and *Thermomyces lanuginosus* representing Ascomycota. The tested species have different repertoires of CAZymes encoded in their genomes, which consequently results in different expression profiles. *Rhizomucor pusillus* and *Thermomyces lanuginosus* possess substantially fewer CAZyme genes than the other three species. For *Rhizomucor pusillus*, the expression of CAZyme genes is very low in both substrates, with the exception that one pectinase gene is overexpressed in alfalfa. The expression of xylanase genes in *Thermomyces lanuginosus* is higher in barley straw than alfalfa straw while the expression of cellulase genes is similar for both substrates. For *Mycothermus thermophilus* genes encoding cellulases were expressed at similar levels in both barley and alfalfa, with higher expression level of xylanase genes in barley and pectinase genes in alfalfa. For *Myceliophthora thermophila*, xylanase genes are highly induced by barley straw while pectinase genes are upregulated by alfalfa straw. *Trametes versicolor* expresses genes encoding cellulases, xylanases and mannanases at similar levels upon growth in barley and alfalfa, with higher expression of pectinase genes when grown on alfalfa. These results suggest that the expression of CAZyme genes in response to complex substrates is complex and can vary appreciably between closely related species with similar genomic content.

197. Thermophilic fungi for efficient plant biomass degradation. Joost van den Brink, Benjamin Stielow, Ronald de Vries. CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.

A major challenge in the sustainable production of biofuels and biochemicals is efficient enzymatic conversion of plant biomass into monomeric sugars. Most enzyme mixtures are currently produced by a small selection of fungal species (e.g. *Trichoderma reesei*, *Aspergillus niger*). However, the fungal kingdom holds many more fungal species which produce enzyme mixtures with beneficial characteristics such as high (hemi-)cellulase activity and high thermostability. *Sordariales* is one of the few fungal orders with thermophilic isolates, of which many have been associated with the production of thermostable enzymes. The goal of our study is to assess the diversity within *Sordariales* for efficient plant biomass degradation. *Sordariales* isolates showed a large variety of optimal growth temperatures ranging from 25°C till 45°C. Phylogenetic analysis, using a novel highly multiplexed targeted next generation sequencing approach, revealed that optimal growth temperature is a polyphyletic trait within *Sordariales* with separate mesophilic, thermotolerant and thermophilic clades. Four thermophilic clades were clearly distinguished: *Myceliophthora* species, *Thielavia terrestris*, *Chaetomium thermophilum*, and *Mycothermus thermophilus*. Interestingly, enzyme characteristics followed the optimal growth temperatures and were a strong polyphyletic trait. Thermophiles within *Myceliophthora* showed the most potential as efficient plant biomass degraders. Especially *M. heterothallica* had good growth on a large range of substrate and was able to produce offspring with a large physiological and genetic variety. Mating and evolutionary engineering strategies were used to further improve *M. heterothallica* capability to degrade biomasses such as sugarbeet pulp and spruce. In conclusion, this study showed the strategic strength of combining fungal diversity with specific selection strategies to find enzyme mixtures with interesting industrial properties.

198. Gapless genome sequence of *Botrytis cinerea* strain B05.10. Jan van Kan[1], Gabriel Scalliet[2]. 1) Lab Phytopathology, Wageningen Univ, Wageningen, Netherlands; 2) Syngenta Crop Protection Münchwilen AG, CH-4332 Stein, Switzerland.

Through a combination of sequencing technologies (Illumina, PacBio), we have assembled a gapless genome sequence of *Botrytis cinerea* strain B05.10, which is corroborated by an optical map. The assembly comprises 18 chromosomes, of which 10 are full-length from one telomere to the other. Two chromosomes are very small (208 and 247 kbp). RNAseq information from a wide range of developmental stages and culture conditions was included in gene prediction pipelines, which permitted to improve gene models significantly and (partly) annotate splice variants. RNASeq analysis revealed non-coding transcripts in intergenic regions. The previously unresolved structure of some secondary metabolite gene clusters was determined (e.g. the botcinic acid cluster, which is important in virulence). Several transcripts appear to have an operon structure, with a single mRNA encoding two proteins. Large scale proteomics was performed to experimentally assess the quality of current gene models and compare it with former versions of the *B. cinerea* genome.

Genetic characterization was performed of a progeny of ~70 individuals from a cross between B05.10 and a field isolate, using Illumina resequencing. A high density linkage map was obtained on which multiple fungicide resistance factors could be mapped. One translocation was observed between the two strains used in the cross. Comparison of genetic with physical distances enabled to identify regions with low recombination rates as well as potential recombination hotspots.

This novel, near-finished genome sequence is a major step forward for the community and provides a valuable resource for future research on *B.cinerea*. A community annotation effort will be organized with support from EBI in the framework of the Ensembl Fungi platform. Community members are invited to participate in the process by adopting chromosomes for curation and annotation.

199. Genetic basis of *Debaryomyces hansenii* killer toxin. Rhaisa Crespo, Heather Hallen-Adams. Food Science and Technology, University of Nebraska, Lincoln, NE.

Yeasts can produce toxic proteins or glycoproteins, known as killer toxins, that are able to kill sensitive yeast species. The production of killer toxins, first discovered in *Saccharomyces cerevisiae*, is a phenomenon known in more than 100 yeast species. *Debaryomyces hansenii* is commonly found in saline environments and has been found to be able to produce these toxins against *Candida albicans* (SC5314) and *Candida tropicalis* (10985) at optimum conditions of 20°C and pH 4.5. The genetic basis and mechanism of action of these toxins is unknown. Cycloheximide treatment of *D. hansenii* prior to or during killer activity assays on methylene blue plates seeded with *C. albicans* and *C. tropicalis* does not reduce activity, suggesting that the toxin is not cytoplasmically-encoded (as are several characterized yeast killer toxins), but chromosomal. To understand the nature of these toxins, 2x250 paired end Illumina sequencing averaging 100x coverage (range: 48x - 145x) was performed on 7 killer strains and 3 non-killer strains of *D. hansenii* previously isolated from different type of cheeses. Numerous differences between *D. hansenii* strains were identified, and will be further analyzed and compared to the published genome strain, *D. hansenii* CBS 767.

200. A *Candida albicans* population genomics study. Marie-Elisabeth Bougnoux[1,2], Katja Swartz[3], Corinne Maufrais[1], Natacha Sertour[1], Guillaume laval[1], Kerstin Voelz[4,5], Robin May[4,5], Richard Bennett[6], Gavin Sherlock[3], Christophe d'Enfert[1,2]. 1) Institut Pasteur, Paris, France; 2) INRA, USC2019, Paris, France; 3) Stanford University, Department of Genetics, School of Medicine, Stanford, California, USA; 4) University of Birmingham, Institute of Microbiology & Infection and the School of Biosciences, Birmingham, UK; 5) NIHR Surgical Reconstruction and Microbiology Research Centre, University Hospitals of Birmingham, NHS Foundation Trust, Queen Elizabeth Hospital, Birmingham, UK; 6) Brown University, Department of Molecular Microbiology & Immunology, Providence, Rhode Island, USA.

Candida albicans is responsible for the majority of life-threatening fungal infections occurring in hospitalized patients and is also the most frequently isolated fungal commensal of humans. The *C. albicans* population includes at least 18 phylogenetic groups (or clades). Specific phenotypes can distinguish isolates within a given clade from those in other clades and yet, the relationships between *C. albicans* natural genetic and phenotypic diversities have not been explored in depth. We have sequenced the diploid genomes of 150 *C. albicans* isolates (100 bp Illumina paired end reads, >50X average sequencing depth) selected from a collection of commensal/clinical isolates previously used to characterize the population structure and belonging to the 12 major *C. albicans* clades. Sequencing of this panel of isolates uncovered more than 500,000 polymorphic positions in the 2x13 Mb *C. albicans* diploid genome. These polymorphisms most often showed clade-specificity and our analyses confirmed the predominantly clonal reproduction of *C. albicans*. Moreover, aneuploidies were rare in our set of sequenced isolates and did not correlate with antifungal resistance. While loss-of-heterozygosity (LOH) events were pervasive across the sequenced isolates, few hot spots of LOH were identified. LOH are a known cause of microevolution in *C. albicans* and our results suggest that they result from independent double-strand breaks (DSB) experienced by individual isolates, as a consequence of normal metabolitic activities or environmental insults such as exposure to antifungals or host attack.

201. Cell-Cycle Transcription Factors in Fungi. Anne Augustus[1,2], Joshua Schipper[2], Raluca Gordan[2], Nicolas Buchler[1,2]. 1) Biology, Duke University, Durham, NC; 2) Center for Genomic and Computational Biology, Duke University, Durham, NC.

Control of the cell cycle is of fundamental importance to all organisms, both single-celled and multicellular. A complexly regulated network of proteins are required to perform all the necessary steps at the right time and in the right order. The large number of genes whose expression is dependent on the cell cycle rely on specific transcription factors (TFs) to turn them on and off. In most eukaryotes, TFs of the E2F/DP family are responsible for this regulation. However, commonly studied model fungi such as *Saccharomyces cerevisiae* lack E2F/DP homologs and use the unrelated SBF/MBF family instead. Phylogenetically, it appears that E2F/DP was the ancestral regulator, as homologs are widespread among sequenced eukaryotes. In contrast, SBF/MBF family members occur only in fungi. Furthermore, the DNA-binding domain they contain, the KilA-N or APSES domain, primarily occurs in bacteria and viruses, not other eukaryotes. This suggests that the SBF/MBF proteins may derive from horizontal gene transfer, perhaps through an ancestral bacterial or viral infection. Intriguingly, many basal fungi have components of *both* regulatory pathways, reflecting a stage in fungal evolution where the SBF/MBF system had been acquired, but hadn't yet replaced the E2F/DP system. We are currently looking at the chytrid *Spizellomyces punctatus* as a representative model for these basal fungi. The DNA-binding domains from the homologous proteins have been cloned and expressed in *Escherichia coli*. We plan to use these purified proteins to characterize the DNA-binding specificity of the transcription factors and determine their regulons. This is the first step in understanding how these two TF families coordinate cell cycle regulation in the basal fungi, and presumably the fungal ancestor that first contained both of them.

202. Genome-wide identification of target genes of a mating-type α-domain transcription factor reveals functions beyond sexual development. K. Becker[1], C. Beer[1], M. Freitag[2], U. Kück[1]. 1) General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic penicillin, the most commonly used drug in the

treatment of bacterial infections. Recently, a functional *MAT1-1* locus encoding the α-box transcription factor MAT1-1-1 was discovered to control sexual development in *P. chrysogenum*. As only little was known from any organism about the regulatory functions mediated by MAT1-1-1 on a genome-wide level, we applied chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq), one of the most powerful tools for genome-wide profiling of DNA-binding proteins.

We present the first application of ChIP-seq for the functional characterization of a transcription factor from *P. chrysogenum*, and, more importantly, the first genome-wide analysis focusing on unraveling the transcriptional regulatory network controlled by a mating-type locus-encoded transcription factor. While MAT1-1-1 has been described as a regulatory protein restricted to the orchestration of sexual reproduction, our data provides strong evidence for mating-type transcription factor functions that reach far beyond their previously understood role in sexual development. These new roles include regulation of hyphal morphology, asexual development, as well as amino acid, iron, and secondary metabolism.

Furthermore, bioinformatics analysis and downstream analysis, including *in vitro* DNA-protein binding studies (EMSA), yeast one-hybrid, and DsRed reporter gene assays in *P. chrysogenum*, enabled the identification of a MAT1-1-1 DNA-binding motif, which shows a high degree of conservation within filamentous ascomycetes. Our studies pave the way to a more general understanding of these master switches for development and metabolism in all fungi, and open up new options for optimization of fungal high production strains. Besides, they will also be relevant for other filamentous fungi, where mating-type loci have already been identified, but so far little is known about their regulatory functions on a genome-wide level.

203. Gene family expansions associated with host range in *Colletotrichum* pathogens. Riccardo Baroncelli[1,2], Serenella A. Sukno[2], Eric Holub[1], Richard Harrison[3], Surapareddy Sreenivasaprasad[4], Michael R. Thon[2]. 1) University of Warwick, School of Life Sciences, Wellesbourne, UK; 2) Universidad de Salamanca, Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Salamanca, Spain; 3) East Malling Research, East Malling, Kent, UK; 4) University of Bedfordshire, Department of Life Sciences, Luton, UK.

Colletotrichum acutatum sensu lato (CAsl) includes a number of important plant pathogens infecting both domesticated and wild plant species worldwide. Members belonging to this complex are able to develop different types of interactions with plant hosts including biotrophic, necrotrophic and hemibiotrophic infections. We sequenced and annotated the genomes of four representative strains, chosen based on their wide host range and phylogeographic position. The genome sequences were used for a wider *Colletotrichum* comparative genomics with the aim of investigating gene family expansions and evolution of candidate effector encoding genes. The four CAsl proteomes along with the proteomes of an additional 6 *Colletotrichum* spp. and 7 other Sordariomycetes were classified into protein families using a variety of tools and were also clustered into groups of homologous proteins. CAsl encode for the highest number of enzymes involved in the degradation of plant cell wall such as xyloglucan, xylan, pectin and cellulose. CAsl are also characterized by the broadest arsenal of secreted peptidases such as metallo and serine peptidases, both families with known roles in plant pathogenesis. Expansions of gene families encoding for degradative enzymes reflect the evolutionary adaptation of CAsl to different hosts and niches and open new opportunities for the identification of novel genes for industrial purposes. Examination of protein domain and family content revealed an expansion in NEPs with CAsl carrying twice the number of the genes compared to other fungi. The expansion of CSEPs appears to be more related to evolutionary history rather than host range. Identified CSEPs specific for species and complexes will facilitate the functional analysis of *Colletotrichum* pathogenicity determinants and should prove useful tools in the search for plant quantitative disease resistance components active against antrachnose.

204. Chromosomal characterization of *Colletotrichum lindemuthianum* and *Glomerella* spp. strains isolated from common bean. P.R.C. Carvalho, R.C. Pereira, M.O. Falleiros, S.F. Mota, E.A. Souza. Universidade Federal de Lavras, Brazil.

Isolations made from anthracnose lesions have shown the presence of asexual colonies of *C. lindemuthianum*, as sexual colonies of *Glomerella* spp. These strains were investigated by morphological, cytological and molecular characterization, as well by infection analysis, and the results suggested that *Glomerella* spp. is a new species co-infecting anthracnose lesions, and not the teleomorph of *C. lindemuthianum*, as was believed until recently. Thus, the characterization of the chromosome set is important, not only for providing information about the genome of the pathogen, but also to identify the existence of chromosomal polymorphism in these different strains, contributing to the characterization of the species, the understanding of the pathogenicity, as well in phylogenetic studies, especially in the species complex of the genus *Colletotrichum*. For the identification of polymorphism in the number and structure of chromosomes have been used the GTBM method. Results have showed that there is chromosomal polymorphism between and within species and strains of *C. lindemuthianum* and *Glomerella* spp, an information that can contribute to basic and applied studies of molecular biology and genome organization of these important plant pathogens.

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205. Evolution of an outbreak: Hypermutators and the *Cryptococcus gattii* outbreak. R. Blake Billmyre[1], Shelly Clancey[1], Sheng Sun[1], Piotr Mieczkowski[2], Joseph Heitman[1]. 1) Duke University, Durham, NC; 2) University of North Carolina, Chapel Hill, NC.

Over the past fifteen years, an ongoing outbreak of the human fungal pathogen *Cryptococcus gattii* has occurred in the Pacific Northwest of the United States and Canada. This outbreak is comprised of three subtypes of the VGII molecular type of *C. gattii*, based on multilocus sequence typing, including the VGIIa/major, the VGIIb/minor, and the VGIIc/novel lineages. We performed whole genome sequencing and analysis of previously published genomes to analyze a total of 53 VGII isolates. Each of the clonal lineages underwent sexual recombination in the past, but recent crosses did not appear to contribute to the establishment of the outbreak lineages. Instead we found that VGIIa and VGIIb were likely introduced independently from South America and Australia respectively, while VGIIc may have arisen

locally. Interestingly, we found that the VGIIa/major component of the outbreak has a clonal sublineage with diminished virulence that harbors a single base deletion in the coding region of the gene encoding the DNA mismatch repair component Msh2. Strains with this nonsense mutation in *MSH2* have an increased mutation rate, ~5-fold in typical genes, but an even more dramatic hypermutator phenotype (~100-fold elevation) in genes containing a homopolymer run within their coding regions. These genes occur frequently in the *C. gattii* genome, with 4% of the gene set containing a homopolymer run of 8 bases or longer in the coding region. One of these mutations in a homopolymer run resulted in unselected drug resistance to both FK506 and rapamycin via inactivation of the gene encoding the FKBP12 homolog. In a *de novo* deletion of *MSH2* and in crosses the hypermutation trait segregates with the *msh2* mutation. We hypothesize that the VGIIa/major strains responsible for the majority of the Pacific Northwest outbreak may have undergone microevolution mediated by a transient hypermutator state in adapting to a new environment to cause disease. This model has been previously demonstrated in virulences trajectories of bacterial pathogens and also operates in human colon cancer tumors but has not been previously observed in a fungal pathogen. Studies in progress seek to address whether the mutator state is an ancestral or derived lineage.

206. Investigating *Cryptococcus neoformans* polysaccharide capsule trafficking with functional genomics. Morgan Wambaugh, Steven Denham, Jessica C. S. Brown. Pathology Dept., Division of Microbiology and Immunology, University of Utah, Salt Lake City, UT.

Cryptococcus neoformans is an opportunistic basidiomycetes pathogen that is estimated to cause ~1 million cases and 600,000 deaths annually worldwide. A key virulence trait in *C. neoformans* is a large, inducible polysaccharide capsule that facilitates immune system evasion early in the infective process. Acapsular mutants are attenuated for virulence. Despite the importance of capsule and its primary polysaccharide, GXM, how GXM is trafficked to and transported across the plasma membrane and cell wall is poorly understood.

We previously used cross-species genomic profiling[1] and high-throughput chemical-genetics[2] to investigate *C. neoformans* gene functions. For cross-species genetic interaction mapping, we expressed genes in *Saccharomyces cerevisiae*, generated a genetic interaction map using the *S. cerevisiae* knockout library, then compared these data to extensive published datasets to generate function predictions, which we then tested in *C. neoformans*. One of our test genes was *LIV7*, a *C. neoformans* virulence gene that lacks an *S. cerevisiae* ortholog. Our cross-species genomic profiling experiments demonstrated that Liv7 interacts genetically with TRAPP complex member Trs33 and localizes to the Golgi; double mutants lack surface GXM. Here we test the hypothesis that Liv7 acts as a member of the TRAPP complex that is involved in GXM trafficking to the cell surface. Our chemical-genetic profiling experiments identified a number of genes involved in the maintenance of capsular polysaccharide GXM on the cell surface. Mutants that produce GXM but do not maintain it on the cell surface are attenuated for virulence in a mouse model of cryptococcosis. We are investigating the molecular function of these genes and their regulatory network.

[1]Brown and Madhani (2012). *PLoS Genetics* 8(12). [2]Brown *et al.* (2014). *Cell* 159(5).

207. Lineage specific genes in *Cryptococcus gattii* respond to oxidative stress and import into the mitochondrial inner membrane. Rhys Farrer[1], Sharadha Sakthikumar[1], Chris Desjardins[1], Sharvari Gujja[1], Yuan Chan[2], Kerstin Voelz[3], Joseph Heitman[2], Robin May[3], Matthew Fisher[4], Christina Cuomo[1]. 1) Genome Sequencing and Analysis Program, The Broad Insititute, Cambridge, MA, United States; 2) Duke University Medical Center, Durham, North Carolina, United States; 3) University of Birmingham, Birmingham, United Kingdom; 4) Imperial College London, London, United Kingdom.

We have analyzed genome wide variation between four known lineages of the pathogenic yeast *Cryptococcus gattii* (VGI, II, III & IV) using *de novo* assemblies for 16 new genomes, including the first representatives of VGIII and VGIV. Synteny across the four lineages is highly conserved, with only 16 structural variants identified that were predominantly found among the VGI-III-IV species cluster. Using synteny to improve orthology detection, we identified 737 Lineage-specific and pan-lineage-specific genes, which were highly syntenic among isolates of that lineage, and dispersed across the genome. Lineage-specific genes are mostly involved in DNA-mediated transpositions, responses to oxidative stress and import into mitochondrial inner membrane. By combining the 16 new genomes with 37 additional whole genome datasets, we found evidence for recent transcontinental spread. Finally, we identified a number of multi-drug transporters undergoing positive selection along multiple branches of VGII and VGIII. Genes responding to nitric oxide stress were also under selection in one VGII branch. Our results reveal the extent of genotypic and phenotypic variation within this species-complex.

208. Phylogenetically-informed and functional identification of the targets for rational design of the next-generation antifungal agents. Giuseppe Ianiri[1], Alexander Idnurm[2]. 1) School of Biological Sciences, University of Missouri-Kansas City; 2) School of Botany, University of Melbourne.

There are limited numbers of antifungal agents currently in use to treat human mycoses and plant diseases. Resistance to these agents can occur and this is a concern for their long-term use. Genes that are essential for cell viability represent preferable targets to develop new antifungal agents. All “essential” genes have been identified in two ascomycete yeast species, *Saccharomyces cerevisiae* and *Schizosaccahromyces pombe*. *Cryptococcus neoformans* is a basidiomycete that causes cryptococcosis disease in immunocompromised and healthy individuals. As a basidiomycete, it diverged from pathogenic or model ascomycete species more than 500 million years ago. A method was developed to identify *C. neoformans* genes that are essential for viability, with both forward and reverse genetics approaches, using an engineered diploid strain and genetic segregation after meiosis. The forward genetic approach generated T-DNA insertional mutants in the diploid strain, the strains were induced to undergo meiosis and sporulation, and a system employed to select for haploid cells with counter selection of the insertion event. More than 2,500 mutants were analyzed, and T-DNA insertions in genes required for viability were identified. For targeted gene replacement, the *C. neoformans* homologs of 35 genes required for viability in ascomycete fungi were disrupted, meiosis and sporulation induced, and the haploid progenies evaluated for their ability to grow on selective media. This analysis

more than doubled the number of known conserved essential genes in the fungi. Genes that have no mammalian or plant homologs and are essential in both basidiomycetes and ascomycetes can be prioritized for the development of antifungal drugs with broad-spectrum activity.

209. Diversification of gene families involved in fruiting body development in Agaricomycetes inferred using comparative genomics. Samuel Kovaka[1], Alicia Knudson[1], Laszlo Nagy[2], John Gibbons[1], David Hibbett[1]. 1) Biology Dept., Clark University, Worcester, MA; 2) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, HAS, Szeged 6726 Hungary.

Agaricomycetes possess complex fruiting bodies, such as gilled mushrooms, as well as simple crust-like corticioid forms. We are studying the evolution of gene families involved in fruiting body development using comparative genomics. We first focused on genes encoding proteins involved in light signaling, which is critical to development in Coprinopsis cinerea and other species. Analysis of "blind" mutants of C. cinerea have identified two genes, dst1 and dst2, that encode proteins involved in blue light signaling; dst1 encodes a homolog of the white collar-1 (wc-1) gene of Neurospora crassa. WC-1 and a second protein, WC-2, form the "white collar complex" (WCC), which functions as both a photoreceptor and transcription factor. The dst2 gene of C. cinerea encodes a protein with a putative FAD-binding domain that may play a role in perceiving blue light. Using BLAST and phylogenetic analyses of data from the genomes of 189 fungi, including 55 Agaricomycetes, we determined that most fungal genomes contain one copy each of wc-1/dst1 and wc-2, except yeasts (Saccharomycotina), chytrids, and zygomycetes (which all lack fruiting bodies). In contrast, all but one of the Agaricomycete genomes contains one copy of dst2, but only one other fungal genome (Gonapodya prolifera) possesses a homolog. The Agaricomycete Piriformospora indica has a highly divergent dst2 sequence, but it is an endophyte that is not known to produce a fruiting body. We infer that light-dependent fruiting body development in Agaricomycetes is a conserved process that is mediated by WCC, which is retained in most filamentous Dikarya, and dst2 , which is restricted almost entirely to Agaricomycetes. We are also using published transcriptomic data from fruiting bodies and mycelia of C. cinerea, Schizophyllum commune, and other species to identify genes that are upregulated in fruiting bodies of multiple species. With this information in hand, we will query genomes from across the fungi to identify gene families in which expansions and contractions are correlated with gains and losses of complex forms in Agaricomycetes.

210. Screening for secondary metabolite gene clusters in novel fungal isolates. Pradeep Phule, Frank Kempken. Abt. Genetische Botanik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Kiel, Germany.

Genes involved in the production of secondary metabolites in fungi are clustered and located adjacent to one another. Pathway specific regulatory genes are embedded in gene clusters (1). Genome mining has revealed that the number of secondary metabolite gene clusters based on conserved PKS and NRPS encoding genes are greater than anticipated. Activation of silent gene clusters may led to the production of novel biomolecules (2).

We are interested into finding new secondary metabolite gene clusters in novel fungal isolates of different ecological niches. We collected 27 soil samples from the botanical garden in Kiel, Germany, and several marine samples from the beaches of the Baltic Sea at Kiel and Eckernförde, Germany. We isolated 20 different fungal strains from Kiel botanical garden, and 15 different fungal strains from Baltic Sea. Six out of 15 strains may represent marine fungi based on growth rate on salt containing media. DNA of these samples was isolated to amplify ITS region and Sanger sequencing was used to identify strains. Selected fungal strains from soil and marine origin have been subjected to next generation sequencing, either by sequencing of DNA from single strains or pooled genomic DNA from several strains. Currently bioinformatics analysis is being performed and putative secondary metabolite genes are being annotated.

211. The interactomes of competeing fungi during wood decomposition succession. Daniel C Eastwood[1], Suzy Moody[1], Jennifer Hiscox[2], Melanie Savoury[2], Ed Dudley[1], Hilary Rogers[2], Carsten Muller[2], Lynne Boddy[2]. 1) Swansea University, Swansea, United Kingdom; 2) Cardiff University, Cardiff, United Kingdom.

Wood decomposition is a critical process in nutrient recycling within forests systems and has wider implications for the global carbon cycle. Decay is driven predominanly by Agaricomycete fungi that have specialised to breakdown recalcitrant lignocellulose. Fungi must balance decomposition and substrate utilisation with continuing growth and foraging, sporulation and competition. The outcomes of competitive interactions are varied with some species exhibiting a more agressive growth than others. Interaction outcomes are also influenced by physical parameters, such as temperature and water availability, and the potential impacts of climate change on these systems is unknown. This ongoing study investigates the transcriptomic and proteomic responses of intermingling and competing decay fungi growing through beech wood blocks during a time course and under different physical conditions.

212. Coupling evolutionary dynamics of *Venturia inaequalis* effectors and functional genomics to decipher mechanisms of virulence and to identify durable resistance genes in apple. Benoit Calmes, Thibault Leroy, Adrien Biessy, Thomas Guillemette, Mélanie Sannier, Pascale Expert, Marie de Gracia, Aurélie Charrier, Jérôme Collemare, Valérie Caffier, Emilie Vergne, Elisabeth Chevreau, Charles-Eric Durel, Christophe Lemaire, Bruno Le Cam. IRHS-INRA, Beaucouzé, France.

During infection, pathogens secrete small secreted proteins (SSPs), called effectors, that promote disease. Plant receptors encoded by resistance *R* genes might recognize such effectors (also called avirulence factors *AVRs*), resulting in plant immunity. Pathogens evade recognition thanks to the emergence of virulent alleles present in populations. It has been demonstrated that avirulent effectors are crucial for the pathogen infection cycle and that their loss-of-function may induce a substantial fitness cost. This kind of effector is expected to be under purifying selective pressure. Here, we aim at identifying the effector repertoire of *Venturia inaequalis*, the agent of apple scab, assessing its evolutionary dynamics and studying the role of candidate effectors in virulence. We sequenced *de novo* 90 strains, collected on apple and on their wild relatives and differing in their host range or virulence to study allelic polymorphism at 880 putative effector loci. The top-20 hits for highly conserved sequences were selected as candidates for further functional analyses. *In planta* gene expression showed a significant induction of these conserved SSP at the early stage of plant infection. Their functions were investigated using targeted deletion mutants. Remarkably, loss of two conserved SSPs resulted in reduced aggressiveness without any alteration in growth *in vitro*.

GFP-tagged protein and heterologous expression were used to assess their sub-cellular localization in infected apple leaves. Involvement of theses SSP in the modulation of host defence was also investigated using an apple full-transcript microarray. Highly conserved effectors will be used to screen for novel *R* genes in *Malus* genotypes characterized for their high resistance to scab. This combined knowledge should enable us to understand strategies used by the pathogen to overcome defences in apple and consequently to build more durable resistance towards apple scab.

213. Comparative transcriptomics analyses used to investigate host-specificity determinants in *Venturia pirina* and *V. nashicola*. Shakira Johnson[1], Nathan Hall[1,2], Jo Bowen[3], Dan Jones[1], Cecilia Deng[3], Vincent Bus[4], Kyungho Won[5], Jang Hoon Song[5], Hideo Ishii[6], Kim Plummer[1]. 1) Botany, La Trobe University, Bundoora, VICTORIA, Australia; 2) Victorian Life Sciences Computational Initiative, Melbourne, Australia; 3) Plant and Food Research, Auckland, New Zealand; 4) Plant and Food Research, Havelock North, New Zealand; 5) National Institute of Horticultural and Herbal Science, Rural Development Administration, Naju, Korea; 6) National Institute for Agro-Environmental Sciences, Tsukuba, Japan.

Pear scab is a major fungal disease of pears worldwide, caused by two distinct species in the genus *Venturia: V. pirina* (causal agent of European pear scab) and *V. nashicola* (causal agent of Asian pear scab). The two fungal species display host specificity, with *V. nashicola* limited to infecting Asian pear (*P. ussuriensis, P. pyrifolia, etc), V. pirina* is limited to infecting European pear (*Pyrus communis).* The incompatible relationship between European pears and *V. nashicola*, and Asian pears and *V. pirina*, is considered to be governed by nonhost resistance; however, the multi layered molecular mechanisms of nonhost incompatibility is poorly understood and the *Pyrus-Venturia* pathosystem provides a unique opportunity to dissect the underlying genetics of this potentially more durable form of resistance. Early defenses from plant hosts in response to attempted infection by a non-adapted pathogen are likely to be governed by recognition of conserved pathogen-associated molecular patterns (PAMPs). This recognition event triggers PAMP-triggered incompatibility (PTI); in adapted pathogens, PTI may be triggered but is soon suppressed by small, secreted proteins from the pathogen, known as effectors. The distinction between the host specificity of *V. pirina* and *V. nashicola* may well be determined by differences in their effector gene arsenal. Genome sequencing technologies have increased the power to perform comparative analyses that assist in the understanding of the molecular mechanisms in pathogenesis. However, effector genes are notoriously difficult to identify using standard gene prediction methods, we have used whole genome and RNA sequencing to reveal unique, host specificity determinants in *V. nashicola* and *V. pirina*..

214. Candidate necrotrophic effectors of the wheat pathogen *Zymoseptoria tritici*. Graeme Kettles[1], Charlie Cairns[2], Ken Haynes[2], Kostya Kanyuka[1], Jason Rudd[1]. 1) Rothamsted Research, Harpenden, UK; 2) University of Exeter, Exeter, UK.

Zymoseptoria tritici (synonym *Mycosphaerella graminicola*) is the most damaging fungal pathogen of wheat in Western Europe. The fungus invades leaves through stomata and grows as extracellular hyphae that lack obvious feeding structures. During this early phase of colonisation, there is minimal induction of conventional plant defence responses. Later in infection, there is a switch to necrotrophy, accompanied by 'defence' gene induction, massive host cell death and nutrient release in infected tissues. Our current model for successful *Z. tritici* infection suggests a variation on the inverse gene-for-gene system characterised for two other wheat pathogens. Namely, after an initial effector-mediated evasion of host responses by stealth, the fungus then deploys other effectors (necrotrophic) which interact with host susceptibility (S) proteins to induce cell death and enhance pathogen colonisation.

To understand the molecular mechanisms underlying *Z. tritici* infection, we have identified 117 candidate secreted protein encoding genes, which show strong expression at key phases of the interaction. Most have characteristics known to be common to effectors identified in other pathosystems, but few have sequence homologues. Using Agrobacterium-mediated transient expression, we identified 15 proteins that induce cell death or chlorosis in the non-host model plant *Nicotiana benthamiana*. Of 11 of these proteins, the majority require localisation to the apoplastic space for their activity. However two proteins retained cell death-inducing activity when expressed intracellularly, suggesting a mode of action requiring internalisation into host cells. We are using heterologous protein expression systems to produce these lead candidates for assessment of cell death-inducing activity in a range of resistant and susceptible wheat cultivars. Our aim is to identify necrotrophic effectors essential for *Z. tritici* virulence on wheat and additionally, uncover the S genes subject to manipulation by the effector repertoire of this pathogen. This project is funded by a fellowship awarded by Rothamsted Research to KK and JJR.

215. Generation of a *ToxA* knockout strain of the wheat tan spot pathogen *Pyrenophora tritici-repentis*. Caroline Moffat, Pao Theen See, Richard Oliver. CCDM, Curtin University, Perth, Australia.

The necrotrophic fungal pathogen *Pyrenophora tritici-repentis* causes tan spot, a major disease of wheat, throughout the world. The proteinaceous effector ToxA is responsible for foliar necrosis on ToxA-sensitive wheat genotypes. The single copy *ToxA* gene was deleted from a wild-type race 1 *P. tritici-repentis* isolate via homologous recombination of a knockout construct. Expression of the *ToxA* transcript was found to be absent in transformants (*toxa*), as was ToxA protein production in fungal culture filtrates. Plant bioassays were conducted to test transformant pathogenicity. The *toxa* strains were unable to induce necrosis on ToxA-sensitive wheat genotypes. To our knowledge, this is the first demonstration of a targeted gene knockout in *P. tritici-repentis*. The ability to undertake gene deletions will facilitate the characterization of other pathogenicity effectors of this economically significant necrotroph.

216. Genome-wide identification of nuclear effectors defines a novel family of pathogenicity factors in *Colletotrichum* spp with host nuclear localization. Walter A. Vargas, José M. Sanz-Martín, Gabriel E. Rech, Michael R. Thon, Serenella A. Sukno. Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, 37185 Villamayor, Spain.

Through a comparative genomic analysis using seven pathogenic fungal species (*Colletotrichum graminicola, Colletotrichum higginsianum, Magnaporthe oryzae, Ustilago maydis, Sporisorium reilianum, Cochliobolus heterostrophus and Fusarium graminearum*), we discovered 263 putative effector proteins likely to target the host nucleus as they simultaneously contain sequence signatures for

secretion and nuclear localization. Using the maize-*Colletotrichum graminicola* pathosystem as a model, we demonstrate that one of the putative proteins, CgEP1 is a novel class of pathogenicity factor. CgEP1 is synthesized during the early stages of disease development and is necessary for fungal hyphae to grow within the epidermal layer of the host. Genetic, molecular and biochemical studies confirmed that this novel effector targets the host nucleus, and is a novel class of DNA-binding protein with regulatory properties that is expressed during pathogenesis. From sequence analysis and allelic variation studies, we discovered that CgEP1 has undergone an intense evolution and selection process that led to duplications of complete internal repeats and has undergone episodes of positive selection. Traces of adaptive evolution were evident in the same codon within each repeat. These residues may be crucial for CgEP1-target binding/recognition and thus relevant for the arms race between host and the pathogen. This effector family is also highly conserved in other monocot-infecting *Colletotrichum* species, affecting important crops such as sorghum, wheat and sugar cane. Our work functionally demonstrates the impact of effectoromics in the identification of novel/unknown effector proteins in fungal species, and their potential use in the development of novel strategies for crop disease management.

217. Effector genes in cucurbit-infecting strains of *Fusarium oxysporum*. Peter van Dam, Sarah M. Schmidt, Martijn Rep. Molecular Phytopathology, University of Amsterdam, Amsterdam, the Netherlands.

The species complex *Fusarium oxysporum* (Fo) represents one of the most abundant and widespread microbes of the soil microflora, and includes plant-pathogenic strains that each have a very narrow host range. Essential for avoiding PTI responses and manipulating host defences, and thereby host specificity, are effector proteins. We wished to find out whether infection of related plant species requires a related set of effector genes in *F. oxysporum*. We therefore generated genome sequences of 32 strains representing four cucurbit infecting *formae speciales* (Foc, Forc, Fon and Fom) and transcriptomes of 2 isolates per *forma specialis*, both *in vitro* and *in planta*.

A transposable element, “miniature impala” (mimp), is always present in the promoter of Fo effector genes, which provides a way to identify new putative effectors from Fo genome sequences. By screening a region downstream of this mimp for ORFs that encode a signal peptide (SP), we generated lists of candidate effector genes in the newly sequenced strains. When looking within a *forma specialis*, a clear resemblance in the presence and sequence of certain putative effector genes was observed, suggesting that some of these genes are responsible for the ability of the strain to infect a specific host. Also between cucurbit-infecting *formae speciales*, there is overlap in effector content, pointing to effectors that may be essential for infection of cucurbits in general. This set of genes forms the basis of our investigation of the molecular basis of host-specificity and host immunity in Fo-cucurbit interactions.

218. Genomic mechanisms accounting for the adaptation to parasitism in nematode-trapping fungi. Tejashwari Meerupati, Karl-Magnus Andersson, Eva Friman, Dharmendra Kumar, Anders Tunlid, Dag Ahren. Department of Biology, Lund University, Lund, Sweden.

Nematode-trapping fungi form unique infection structures, traps, to capture and kill free-living nematodes. The traps have evolved differently along several lineages and include adhesive traps (knobs, nets or branches) and constricting rings. We show, by genome sequencing of the knob-forming species *Monacrosporium haptotylum* and comparison with the net-forming species *Arthrobotrys oligospora*, that two genomic mechanisms are likely to have been important for the adaptation to parasitism in these fungi. Firstly, the expansion of protein domain families and the large number of species-specific genes indicated that gene duplication followed by functional diversification had a major role in the evolution of the nematode-trapping fungi. Gene expression indicated that many of these genes are important for pathogenicity. Secondly, gene expression of orthologs between the two fungi during infection indicated that differential regulation was an important mechanism for the evolution of parasitism in nematode-trapping fungi. Many of the highly expressed and highly upregulated *M. haptotylum* transcripts during the early stages of nematode infection were species-specific and encoded small secreted proteins (SSPs) that were affected by repeat-induced point mutations (RIP). The high expression and rapid divergence of SSPs indicate a striking similarity in the infection mechanisms of nematode-trapping fungi and plant and insect pathogens from the crown groups of the filamentous ascomycetes (Pezizomycotina). The patterns of gene family expansions in the nematode-trapping fungi were more similar to plant pathogens than to insect and animal pathogens. The RIP activity in the Orbiliomycetes suggested that this mechanism was present early in the evolution of the filamentous ascomycetes.

219. Whole genome comparisons in *Fusarium oxysporum* spp. reveal a three-speed genome, large-scale losses and putative horizontal transfer events. Like Fokkens, Peter van Dam, Sarah Schmidt, Martijn Rep. Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands.

In many pathogen genomes, genes that are involved in pathogenicity cluster with transposable elements (TEs) in fast-evolving genomic regions: a two-speed genome. *Fusarium oxysporum* strain *Fol4287* has 11 conserved core chromosomes and 4 lineage specific (LS) pathogenicity chromosomes. This provides an explanation for the polyphyletic nature of host specificity in *F. oxysporum.* One of the LS chromosomes has been shown to transfer horizontally and convey pathogenicity from *Fol4287* to a non-pathogenic strain. To determine the extent to which horizontal chromosome transfer (HCT) and other genetic processes shaped evolution of pathogenicity in *F. oxysporum*, we construct whole genome alignments between 32 published and newly sequenced *F. oxysporum* strains. First we compared the level of sequence and synteny conservation for different *Fol4287* chromosomes. We find that *Fol4287* has a three-speed rather than a two-speed genome: the smallest three core chromosomes (11, 12 and 13) show more sequence divergence and loss of synteny than the other core chromosomes. Moreover, one cucumber-infecting strain lost chromosome 12 entirely, and one melon-infecting strain lost almost half of chromosome 13, indicating that, surprisingly, these chromosomes are (partly) dispensable. The LS chromosomes in *Fol4287* are confined to one clonal line with the exception of the mobile ‘pathogenicity’ chromosome that is also present in other clonal lines infecting tomato. This suggests that HCT was involved in evolution of host specificity in *Fol*. We then also surveyed sequences that did not align to the *Fol4287* reference genome. We find that most genomes have scaffolds that are largely absent in other strains. By comparing presence/absence patterns of genomic regions along the phylogenetic tree we are able to predict virulence and host preference for different

strains. We will subject informative genomic regions to more detailed analyses to identify virulence genes and assess the role of horizontal transfer in the spread of host-specific pathogenicity.

220. *Epichloë* endophytes: a powerful model system for determining genomic responses to genome merger. Austen Ganley[1], Murray Cox[2]. 1) INMS, Massey University Auckland, New Zealand; 2) IFS, Massey University Palmerston North, New Zealand.

Allopolyploidy results from the union of two different parental genomes, producing a new species with double the chromosome complement. Allopolyploidy has been documented in many eukaryotic lineages, and is a major force in evolution. Although it has long been recognized that allopolyploids must overcome a suite of biological responses to genome merger, termed "genome shock" by McClintock, until recently we lacked the means to determine on a genome-wide basis how they overcome this shock. We are employing the *Epichloë* endophytes as a model system to study genomic responses to allopolyploidy. These fungi form associations with grasses, and a number have been shown to be allopolyploids. As with other fungi, their genomes are relatively small and thus amenable to next-generation sequencing approaches, a feature we are currently exploiting to investigate several genomic responses to genome merger. We have shown using RNA-seq that the transcriptional responses to allopolyploidy are predominantly conservative: both copies of most genes are retained; over half the genes inherit the gene expression patterns seen in the parental species; and parental differential expression is often lost in the allopolyploid. These patterns of gene expression change are extraordinarily concordant with those seen in cotton. The very different nature of these two allopolyploids implies a conserved, eukaryote-wide transcriptional response to genome merger. We propose a model in which the cross-kingdom conservation in transcriptional response reflects the mutational processes underlying eukaryotic gene regulatory evolution. We will also present results comparing in culture transcriptome analyses to those done with the fungus *in planta*. To enable other researchers to conduct similar analyses, we developed an easy-to-use software tool, HyLiTE (http://hylite.sourceforge.net), that uses a robust statistical framework to allocate allopolyploid RNA-seq reads to the two (or more) parents, even if a reference genome sequence is not available. This work is part of a suite of analyses we are undertaking to determine genomic responses to allopolyploidy, and it highlights the utility of the *Epichloë* endophytes for studying these responses.

221. Old dogs, new tricks: Investigating the genomics of adaptation to new niches within the *Serpulaceae*. Jaqueline Hess[1], Björn Canbäck[2], Sudhagar Balasundaram[1], Dag Ahrén[2], Håvard Kauserud[1], Alexander Nederbragt[1], Anders Tunlid[2], Tomas Johansson[2], Inger Skrede[1]. 1) Department of Biosciences, University of Oslo, Oslo, Norway; 2) Department of Biology, Lund University, Sweden.

Ecological transitions, for example from a free-living, saprotrophic nutritional mode to one that is dependent on a symbiotic partner, are not uncommon in fungi. Ectomycorrhizal (ECM) symbiosis, for instance, has evolved repeatedly, in phylogenetically distant lineages, and from different nutritional strategies. Comparative genomics has been instrumental in highlighting the commonalities and differences among the genomic architectures of different ECM species. Yet, due to the general plasticity of fungal genomes, hundreds if not thousands of genes may be differentially duplicated or lost between any two species, and often the most pronounced changes involve gene families with broad functional classification such as signaling proteins or proteins containing protein-protein interaction domains. Unsurprisingly, it can be difficult to pinpoint the genes that are most important for success in the new niche. The family *Serpulaceae* (Basidiomycota/Agaricomycota/Boletales) comprises the polyphyletic saprotrophic genus *Serpula* and, nested within, two ECM genera: *Austropaxillus* and *Gymnopaxillus*. While most *Serpula* species are free-living brown rotters, the genus' most well known member, *Serpula lacrymans* is adapted to a life in human-built environments where it aggressively degrades timber construction. Unlike its relatives, *S. lacrymans* is rarely found in the wild and has thus also undergone an ecological transition. We hope that by comparing the signatures of adaptation between the ECM species *A. statuum* and the house-invader *S. lacrymans*, we will be able to begin to distinguish a more general pattern of adaptation to a new environment from those that are specific to their respective ecologies. To achieve this, we have sequenced and assembled the genomes of *A. statuum* and the brown rot species *S. incrassata*, an outgroup to *A. statuum* and *S. lacrymans*. Preliminary data suggests that *A. statuum* has an expanded genome compared to both *S. lacrymans* and *S. incrassata*.

222. Comparative genomics reveal expansions of ABC transporters involved in drug resistance in the mycoparasite *Clonostachys rosea*. Magnus Karlsson[1], Mikael Durling[1], Mukesh Dubey[1], Chatchai Kosawang[2], David Collinge[2], Dan Funck Jensen[1]. 1) Forest Mycology & Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Plant and Environmental Sciences and Copenhagen Plant Science Center, University of Copenhagen, Copenhagen, Denmark.

Mycoparasitic fungi must be able to tolerate toxic secondary metabolites produced by the fungal prey, plants and competing microorganisms. The mycoparasitic fungus *Clonostachys rosea* is an efficient biological control agent (BCA) under field conditions for a variety of plant diseases on agricultural crops. We sequenced the genome of *C. rosea* strain IK726 using Illumina/SOLiD technology, and comparative genomics revealed that *C. rosea* contained the highest number of ATP-binding cassette (ABC) transporters (86) and major facilitator superfamily (MFS) transporters (620) among the studied fungi. Interestingly, the significant ($P \leq 0.05$) increase of ABC transporter gene number in *C. rosea* was associated with phylogenetic groups B (multidrug resistance transporters) and G (pleiotropic drug resistance transporters). A phylogenetic analysis showed that specifically subgroups B-III, G-I and G-V, which all contained characterised members involved in drug resistance, were expanded in ABC transporter gene numbers. *C. rosea* was highly tolerant against mycotoxins and fungicides. We showed that ABC transporter genes *abcG5* and *abcG29* were induced during exposure to the *Fusarium* mycotoxin zearalenone (ZEN) and the fungicides azoxystrobin (only *abcG5*), boscalid, iprodione and mefenoxam, while *abcB3* was induced by boscalid. Deletion of the *abcG5* ABC transporter gene resulted in mutants with reduced growth rate ($P=0.001$) on ZEN-containing media and failure to protect barley seedlings against *F. graminearum* foot rot disease in growth chamber tests. In addition, Δ*abcG5* mutants displayed reduced growth rate on the fungicide mefenoxam and were unable to grow on iprodione, while growth on azoxystrobin and boscalid were not compromised. In summary, our data suggest that membrane transporters partly protect *C. rosea* against xenobiotics, which may find important agricultural applications such as combination treatments of *C. rosea* together with low dose fungicides and other BCAs.

223. Deep origin of Opisthokonta opsin-like GPCRs and bacteriorhodopsins supports opsin convergent evolution. Edgar M. Medina[1], Steve Ahrendt[3], Andrew Swafford[2], Todd Oakley[2], Jason E. Stajich[3]. 1) Department of Biology, Duke University, Durham, NC; 2) Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA; 3) Department of Plant Pathology and Microbiology, University of California, Riverside, CA.

The homology of type-I ("bacteriorhodopsins") and type-II ("animal") opsins has long been debated, with important implications for the origins and history of light sensing, vision, and eyes. While type-I and type-II opsins are often considered convergent, recent studies have resurrected a hypothesis of homology for all opsins, implying that type-I are derived from type-II opsins by a horizontal transfer event. To test this hypothesis, we searched a diverse set of fully sequenced genomes, including chytrid fungi and other divergent opisthokonts to identify opsin-like sequences for phylogenetic analyses, including estimation of divergence times. Although opsin-like GPCRs were present in the opisthokont ancestor and have persisted in the chytrids, type-II opsins originated about 625 million years ago, in the common ancestor of bilaterian and cnidarian animals. We also find that multiple type-I opsin lineages may have been acquired by the opisthokont ancestor through an algal intermediary, including a type-I opsin fused to a guanylyl cyclase in chytrid fungi or to a phosphodiesterase in a choanoflagellate. This suggests that type-I rhodopsins and ancestral opsin-like GPCRs coexisted, at least in the ancestor of chytrids, and that type-I opsins predated type-II opsins, refuting the hypothesis that type-I opsins are derived from type-II opsins. If not homologous, then type-I and type-II opsins result from a remarkable and detailed example of convergent evolution.

224. The genomic landscape of early fungal evolution: Genomic innovations in the earliest fungal ancestors. Laszlo G Nagy[1], Robin A Ohm[2], Robert Riley[3], Francis M Martin[4], Igor V Grigoriev[3], David S Hibbett[5]. 1) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC, HAS, 6726 Szeged, Hungary; 2) University of Utrecht, Department of Microbiology, 3584CH Utrecht, The Netherlands; 3) U.S. Department of Energy Joint Genome Institute, Walnut Creek, California 94598, USA; 4) INRA, UMR 1136, INRA-Nancy Université, Interactions Arbres/Microorganismes, 54280 Champenoux, France; 5) Clark University, Biology Department, Worcester, MA 01610, USA.

Fungi possess a plethora of genomic and phenotypic traits that make them unique and economically important among other life forms on Earth. Whereas vast knowledge has accumulated about many of these in extant model systems, the early evolutionary events that established generic fungal traits are hardly known. What are genomic events that lead to the emergence of a unique fungal lineage? What distinguishes fungi from closely related unicellular forms and animals? To address these questions, we reconstructed gene duplication-loss events in 59 fungal and outgroup species for which whole genome sequences have been published and inferred the ancestral genome composition of the last common ancestor (LCA) of Fungi and that of Dikarya. We reconstructed 1935 and 2794 gene duplications on the branches leading to the LCA of Fungi and the LCA of Dikarya, respectively. On the other hand, only 96 gene losses were inferred in the fungal LCA and 1182 in the Dikarya. Genomic innovations in the fungal ancestor are, among others, enriched in protein families related to intracellular transport and chitin metabolism, whereas those in the LCA of Dikarya are enriched in protein families showing oxidoreductase, ion transporter and chitin synthase activities. Notably, several domains of unknown function (DUF) were found to have emerged either in the LCA of Fungi and that of Dikarya, possibly marking as yet uncharacterized fungal-specific protein families. We discuss the results in the context of fungal functional diversification.

225. Genomic evolution of the ascomycetous yeasts. Robert Riley[1], Sajeet Haridas[1], Asaf Salamov[1], Kyria Boundy-Mills[2], Markus Göker[3], Chris Hittinger[4], Hans-Peter Klenk[5], Mariana Lopes[4], Jan P. Meier-Kolthoff[3], Antonis Rokas[6], Carlos Rosa[7], Carmen Scheuner[3], Marco Soares[4], Benjamin Stielow[8], Jennifer H. Wisecaver[6], Ken Wolfe[9], Meredith Blackwell[10], Cletus Kurtzman[11], Igor Grigoriev[1], Thomas Jeffries[12]. 1) US Department of Energy Joint Genome Institute, Walnut Creek, CA; 2) Department of Food Science and Technology, University of California Davis, Davis, CA; 3) Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, GERMANY; 4) Laboratory of Genetics, Genetics/Biotechnology Center, Madison, WI; 5) School of Biology, Newcastle University, Newcastle upon Tyne, UK; 6) Department of Biological Sciences, Vanderbilt University; 7) Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; 8) CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; 9) UCD School of Medicine & Medical Science, Conway Institute, University College Dublin, Dublin, Ireland; 10) Department of Biological Sciences, Louisiana State University, Baton Rouge, LA; 11) USDA, ARS, MWA, NCAUR, BFPM, Peoria, IL; 12) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Yeasts are important for industrial and biotechnological processes and show remarkable metabolic and phylogenetic diversity despite morphological similarities. We have sequenced the genomes of 16 ascomycete yeasts of taxonomic and industrial importance including members of Saccharomycotina and Taphrinomycotina. Phylogenetic analysis of these and previously published yeast genomes helped resolve the placement of species including *Saitoella complicata*, *Babjeviella inositovora*, *Hyphopichia burtonii*, and *Metschnikowia bicuspidata*. Moreover, we find that alternative nuclear codon usage, where CUG encodes serine instead of leucine, are monophyletic within the Saccharomycotina. Most of the yeasts have compact genomes with a large fraction of single exon genes, and a tendency towards more introns in early-diverging species. Analysis of enzyme phylogeny gives insights into the evolution of metabolic capabilities such as xylose fermentation, methanol utilization, and assimilation of alternative carbon sources.

226. Genome plasticity mediated by transposable elements drives the evolution of virulence in the vascular wilt pathogen *Verticillium dahliae*. Michael Seidl, Luigi Faino, David Cook, Xiaoqian Shi-Kunne, Grardy van den Berg, Bart Thomma. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Verticillium dahliae is a soil-borne pathogen that aggressively colonizes hundreds of host plants, including high value crops such as tomato and potato, leading to the formation of vascular wilt disease. Resistance in the host population exert selective pressure on the pathogen forcing the rapid evolution of adaptive traits to successfully participate in the arms race with the host. By comparative genomics of the *V. dahliae* population, we recently revealed genomic rearrangements that facilitate the gain and loss of genetic material and establish highly dynamic lineage-specific (LS) regions. LS regions are enriched for transposable elements (TEs) and *in planta* induced effector genes

encoding secreted protein that significantly contribute to aggressiveness towards the host, and thus have been hypothesized to contribute to the genome plasticity required for adaptive genome evolution. However, the factors that drive genome plasticity in *V. dahliae* remain enigmatic. Using long-read sequencing technologies, we re-sequenced two *V. dahliae* strains and analyzed the previously identified genomic rearrangements in unprecedented detail, revealing multiple genomic breakpoints down to the nucleotide level. Genomic breakpoints are flanked by multiple TEs, suggesting that these elements play essential roles in their formation. Comparative analyses of *V. dahliae* with the recently sequenced non-pathogenic *Verticillium tricorpus* revealed a highly expanded TE repertoire in pathogenic *V. dahliae*, where *in planta* induced effector candidates, but also other genes encoding secreted proteins, are frequently flanked by TEs. Additionally, whole-genome bisulfite sequencing of *V. dahliae* identified DNA methylation predominantly targeting TEs. In fungi, inactivation of TEs by DNA methylation is common, and we hypothesize that it could also influence the expression of nested effector candidates, thereby providing yet another route how TEs can affect the interaction between the pathogen and its host. In summary, we highlight the profound role of TEs on the evolution of virulence in the vascular wilt pathogen *V. dahliae*.

227. Invasiveness of the harmful house-invader *Serpula lacrymans* – population genomics of the Japanese and European populations. I. Skrede[1], J. Hess[1], S. V. Balasundaram[1], D. Eastwood[2], F. Martin[3], A. Kohler[3], C. Murat[3], D. Barry[4], M. Brandström Durling[5], H. Kauserud[1], N. Högberg[5]. 1) Department of Biosciences, University of Oslo, Oslo, Norway; 2) Department of Biosciences, Swansea University, Swansea, UK; 3) Interactions Arbres-Microorganismes, INRA-Lorraine University, Nancy, France; 4) Société Alcina, Montpellier, France; 5) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The dry rot fungus, *Serpula lacrymans*, is the most efficient decomposer of buildings in temperate regions worldwide. Population genetic data indicate that the species invaded Europe and Japan independently from its native range in central Asia. The European population has further dispersed to the Americas and Australia, and both the Japanese and European populations have reached New Zealand and there apparently admixed. In this study we are investigating the genetic, expressional and physiological basis for the success of the fungus as an invader of human-made wood constructions. Whole genome sequences are obtained from twenty strains from each of the two initial founder populations (Europe and Japan). Our preliminary results (based on two genomes from each population) show that the genetic diversity is higher in the Japanese population, as there is a tenfold difference in number of SNP detected within populations. This is also supported by previous studies using microsatellites and mating type linked markers. We also find that the Japanese strains are better competitors than the European strains (in our experimental set-up they displace the competitor in 60% vs. 45% of the encounters, respectively). In addition, preliminary results indicate that the Japanese population is more efficient in decomposing wood than the European population. We suggest that the better performance among the Japanese isolates are linked to the higher genetic variation found in this population.

228. Diverse origins of genes associated with a growth QTL in *Fusarium circinatum*. S. van Wyk[1], B.D. Wingfield[2], L. De Vos[2], N.A. van der Merwe[2], E.T. Steenkamp[1]. 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Fusarium circinatum is the causal agent of pitch canker of *Pinus* species. Previous work on this fungus has shown that mycelial growth rate is controlled by a quantitative trait locus (QTL) that spans 12 kb of the third chromosome. Here we aimed to characterize and infer the evolutionary history of this region. Annotation revealed that the 12 kb region include five genes that are apparently unique to the pitch canker fungus. These genes respectively encode a monocarboxyl substrate transporter belonging to the major facilitator superfamily, two fungal transcription factors and two aminotransferases. Genome comparisons showed that this region was structurally conserved and present in all *F. circintum* isolates investigated. The results of maximum likelihood phylogenetic analyses suggested that four of these genes were likely acquired horizontally from other *Fusarium* species (the aminotransferase genes) or other hypocrealean fungi (the fungal transcription factor genes). An internal duplication (involving a homologue on chromosome 8) in the *F. circinatum* genome probably gave rise to the monocarboxyl substrate transporter gene. Sequence analysis of the *F. circinatum*-specific region further revealed various signatures of the Gypsy superfamily of retrotransposons. Our data thus suggest that the genomic region associated with the growth rate QTL in *F. circinatum* have diverse origins and that its assembly likely involved multiple steps and the action of transposable elements. Future work will utilize recombinant DNA technology to fully characterize the role of this region and its genes in the biology of the pitch canker fungus.

229. The evolutionary history and structure of β-fructofuranosidases in pathogenic and saprophytic members of the *Ceratocystidaceae*. M.A. van der Nest, C. Trollip, T. Hall, E. Sauerman, D. Roodt, P.M. Wilken, M.J. Wingfield, B.D. Wingfield. University of Pretoria, Pretoria, Gauteng, South Africa.

β-fructofuranosidases (glycoside hydrolases family 32) are a group of Carbohydrate-Active enzymes (CAZymes) that hydrolyse the glycosidic bonds of complex saccharides. Fungi rely on these enzymes to gain access to plant-derived sucrose, and more importantly, to metabolize these into the usable forms of glucose and fructose. In fungi, invertase genes are found in higher copy numbers in the genomes of pathogens than when compared to closely related saprobes. This suggests an important association between invertases and ecological strategy. The aim of this study was to investigate the distribution and evolution of invertases in the *Ceratocystidaceae* using a bioinformatics approach. Species in this group of fungi provide interesting examples to study the evolution of these genes because it includes economically important pathogens such as *Ceratocystis fimbriata*, *C. manginecans* and *C. albifundus*, as well as saprobes such as *Huntiella moniliformis*, *H. omanensis* and *H. savannae* (previously also treated in the genus *Ceratocystis*). Our results showed that the pathogenic species investigated all contained two copies of the invertase gene, while the saprobes only had a single copy. The data also suggested that independent gene duplication and possibly retrotransposition played important roles in the evolution of the invertase gene family in these fungi. Furthermore, this research provided insights into the role of CAZymes in plant-fungal interactions and it adds to the

current understanding of the evolution of plant-fungal interactions as a whole.

230. The Evolution of Fungal Metabolic Pathways. Jennifer Wisecaver[1], Jason Slot[1,2], Antonis Rokas[1]. 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Plant Pathology, The Ohio State University, Columbus, OH.

Fungi contain a remarkable range of metabolic pathways, sometimes encoded by gene clusters, enabling them to digest most organic matter and synthesize an array of potent small molecules. In fungal genomes, the genes involved in these metabolic pathways can be physically linked on chromosomes, forming gene clusters. This extraordinary metabolic diversity is integral to the variety of ecological strategies that fungi employ, but we still know little about the evolutionary processes involved in its generation. To address this question, we analyzed 247,202 enzyme-encoding genes participating in hundreds of metabolic reactions from 208 diverse fungal genomes to examine how two major sources of gene innovation, namely gene duplication and horizontal gene transfer, have contributed to the evolution of clustered and non-clustered metabolic pathways. We discovered that gene duplication is the dominant and consistent driver of metabolic innovation across fungal lineages and metabolic categories; in contrast, horizontal gene transfer appears highly variable both across organisms and functions. The effects of both gene duplication and horizontal gene transfer were more pronounced in clustered genes than in their non-clustered counterparts suggesting that metabolic gene clusters are hotspots for the generation of fungal metabolic diversity. Finally, as a case study, we investigated how both gene duplication and horizontal gene transfer have contributed to the evolution of the bikaverin gene cluster found in some species of *Fusarium* and *Botrytis*..

231. Lessons from 455 *Fusarium* Polyketide Synthase's. Daren W. Brown, Todd J. Ward, Robert H. Proctor. Bacterial Foodborne Pathogens and Mycology, USDA/ARS, Peoria, IL.

In fungi, polyketide synthases (PKSs) synthesize a structurally diverse array of secondary metabolites (SMs) with a range of biological activities. The most studied SMs are toxic to animals and/or plants, alter plant growth, have beneficial pharmaceutical activities, and/or are brightly colored pigments. Here, examination of genome sequence data from 30 species of *Fusarium* identified 455 ostensibly functional PKS genes and 85 nonfunctional PKS pseudogenes. In phylogenetic analyses, the functional PKS genes were resolved into the four clades previously described for Type I fungal PKSs. Only three PKSs were present in all 30 species, and although more closely related species tended to share more closely related PKS gene homologs, there is marked variation in the presence and absence of the homologs among closely related species. For example, the PKS required for depudecin synthesis is present in five members of the *Fusarium fujikuroi* species complex that were examined, but absent in the seven other members of this complex. In contrast, 12 PKS genes were unique to one or two of the species examined. These findings suggest frequent independent loss of PKS genes within species complexes (multispecies lineages) of *Fusarium* as well as horizontal transfer of multiple PKS genes between *Fusarium* species. Overall, based on the predicted functional domain content of each of the PKSs as well as their phylogenetic relationships to one another and to other fungal PKSs, we propose that the 455 *Fusarium* PKSs constitute at least 51 distinct functional orthologs that have the potential to synthesize structurally distinct polyketides.

232. Specific expansion and functional importance of a group of Histidine Kinases in a genus of Fusarium. Greg DeIulio, Li-Jun Ma. UMass Amherst, Amherst, MA.

Fungi and their host plants recognize one another on a molecular level. These interactions are mediated by signaling molecules that directly, or indirectly, detect the presence of a pathogen or host plant. Histidine kinases are a group of well conserved signaling receptors that are present in plants, fungi, and bacteria. Previous studies have shown that some histidine kinases are important fungal signaling molecules, having been implicated in osmosensing, reproduction, fungicide resistance, and pathogenicity. We have identified a small group of genes that appear to be upregulated during infection that belong to a group of histidine kinases unique to *Fusarium* species. Here we present preliminary work identifying the divergence and evolution of these histidine kinases in three species of Fusarium.

233. Whole-genome analysis of *Fusarium graminearum* insertional mutants identifies virulence associated genes and unmasks untagged chromosomal deletions. Martin Urban, Robert King, Keywan Hassani-Pak, Kim Hammond-Kosack. Wheat Pathogenomics Team, Plant Biology and Crop Science, Rothamsted Research, Harpenden, UK.

Identifying pathogen virulence genes required to cause disease is crucial to understanding the mechanisms underlying the pathogenic process. Plasmid insertion mutagenesis of fungal protoplasts is frequently used for this purpose in filamentous ascomycetes. Identifying the insertion event has previously met with varying degrees of success, from a cleanly disrupted gene with minimal deletion of nucleotides at the insertion point to multiple-copy insertion events and large deletions of chromosomal regions.

We used a high-throughput whole-genome sequencing (WGS) approach using Illumina HiSeq 2000 technology to investigate DNA tag insertion points and chromosomal deletion events in mutagenised, reduced virulence *F. graminearum* isolates identified in disease tests on wheat *(Triticum aestivum).* A bioinformatics workflow, called FindInsertSeq, was developed to localise the DNA tag insertions to the nucleotide level. The workflow was tested using four mutants showing evidence of single and multi-copy insertions in DNA blot analysis. The FindInsertSeq method was able to identify both single and multi-copy concatenation insertion sites. By comparing sequencing coverage, unexpected molecular recombination events such as large tagged and untagged chromosomal deletions, and DNA amplification were observed in three of the analysed mutants. A random data sampling approach revealed that 22 x fold genome coverage is sufficient to survey *F. graminearum* for genome alterations. In some cases insertion events are accompanied with large untagged chromosomal deletions while in other cases a straight-forward insertion event could be confirmed. The FindInsertSeq analysis workflow presented enables researchers to efficiently characterise insertion and deletion mutants in fungi and is available for download at https://github.com/Rothamsted/Applied Bioinformatics.

Our work is supported by the BBSRC, through the Institute Strategic Programme 20:20 Wheat®.

234. Characterisation of a dispensable chromosome in *Fusarium circinatum*. S. Fouché[1], B.D. Wingfield[2], M.P.A. Coetzee[2], S. Slinski[2], E.T. Steenkamp[1]. 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Fusarium circinatum is the causal agent of the devastating pitch canker disease of *Pinus* species. The fungus is a member of the so-called *Fusarium fujikuroi* species complex. Based on pulsed field gel electrophoresis (PFGE), members of this complex have previously been suggested to harbour twelve chromosomes. It was also shown that the twelfth chromosome typically exhibits extensive size variation among species, and may even be dispensable in some species. Despite the availability of whole genome sequence data for *F. circinatum*, no information is available regarding its twelfth chromosome. Our aim was therefore to study the extent of variation in chromosome 12 of isolates belonging to *F. circinatum.* For this purpose, the twelfth chromosome of an isolate for which genome sequences are available was separated via PFGE, extracted and sequenced using Illumina technology. Following *in silico* assembly of the twelfth chromosome, PCR primers targeting various regions spanning the chromosome were designed. These primers were then used to analyse the presence or absence of specific chromosomal segments in a collection of *F. circinatum* isolates obtained from around the world. Our results revealed a high level of diversity associated with the twelfth chromosome as each isolate was characterized by a unique composition PCR fragments. A similar analysis of the progeny of an experimental cross further indicated that the twelfth chromosome of *F. circinatum* is meiotically unstable and absent in some of the progeny. Our data thus suggest that chromosome 12 is highly variable in size and sequence and that it likely forms part of the rapidly evolving accessory genome of *F. circinatum*. Future work will reveal whether it serves as a hotspot for evolution or whether the chromosome is undergoing a process of degeneration.

235. Dynamics of wheat gene and protein expression during the susceptible response to Fusarium Head Blight. Ludovic Bonhomme[1], Cherif Chetouhi[1], Pauline Lasserre-Zuber[1], Philippe Lecomte[1], Florence Cambon[1], Olivier Soudière[1], David G Biron[2], Thierry Langin[1]. 1) INRA / B. Pascal University, GDEC, Clermont Ferrand, France; 2) CNRS / B. Pascal University, LMGE, Clermont Ferrand, France.

Fusarium graminearum (Fg) is the main causal agent of the Fusarium head blight disease (FHB) in a wide range of staple cereal crops. As a consequence of infection, severe yield and quality losses occur along with in planta mycotoxin (DON) production making grains incompatible with food uses. Our knowledge of the molecular and physiological plant responses to FHB remains still fragmentary, and was mainly focused on the study of the molecular basis of FHB quantitative resistance. Identify the main factors that make wheat susceptible may open the way to the construction of effective and sustainable resistance in fields. A genome-wide transcriptome analysis along with a 2-DE proteomics approach (Chetouhi et al. 2014, *DOI 10.1007/s10658-014-0552-0*) have been used to trace the cellular pathways targeted by Fg and to identify putative susceptibility factors to FHB. Profiling was conducted during Fg-infection process over a 14-day period covering five key stages of grain development of a susceptible wheat cultivar. Our results revealed that Fg infection does not deeply alter both the grain transcriptome and proteome, with about 5% of the whole set of trancripts or proteins that displayed abundance changes. Specific statistical analyses computing both the infection effect and grain ontogeny revealed specific patterns of FHB responses, including grain development-specific changes which suggest that Fg could take advantage of putative susceptibility factors closely related to grain development processes. The first steps of grain ontogeny were slightly impacted but acute molecular remodeling was evidenced during the grain filling stage and putatively connected with DON synthesis. Up to 1300 Fg-responsive genes and 75 regulated proteins were evidenced, including known susceptibility factors. They included defense proteins and others regulators involved in primary metabolism and in cell death reprogramming. Our survey provides new insights into key molecular events controlling the susceptible response to FHB in wheat grains and into new knowledge on putative susceptible genes.

236. *Fg*Not5 is Required for Sexual and Asexual Development, and Temperature Stress Tolerance in *Fusarium graminearum*. Cuong Bui, Hokyoung Son, Yin-Won Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea.

The Ccr4-Not complex has crucial roles in mRNA synthesis and degradation and therefore regulates gene expression at multiple levels. Not3 and Not5 subunits shares similar N-terminal domain required for tolerance to high temperature, while the critical importance of Not5 for vegetative growth maps to its C-terminus in yeast. Like other higher eukaryotes, only one ortholog gene (*FgNOT5*) for yeast *NOT3* and *NOT5* was identified in the head blight fungus *Fusarium graminearum.* In this study, we investigated biological functions of *Fg*Not5 in *F. graminearum.* Deletion of *FgNOT5* resulted in retarded growth, swollen hyphal tips and delayed conidium production compared to the wild-type strain. Approximately 56% of the *ΔFgnot5* conidia had three septa or less, while most of wild-type conidia had more than four septa. Deletion mutant of *FgNOT5* showed increased sensitivity to high temperature similar to yeast. Moreover, the *ΔFgnot5* mutants lost self- and female fertility. Taken together, our results showed that *Fg*Not5 is required for normal growth, asexual and sexual development, and thermal stress tolerance in *F. graminearum*.

237. Dissecting the biology of *Fusarium graminearum* in a high-throughput manner: Competitive fitness assays within barcoded mutant pool. Donald Gardiner, Aurelie Benfield, Kemal Kazan. Agriculture Flagship, CSIRO, St Lucia, Queensland, Australia.

In nature, competition between microbial strains for supremacy on their host or in their environmental niche is the norm. In yeast and a number of bacterial species, competitive interactions are routinely applied to understanding processes such as niche colonisation and resistance to xenobiotics. In contrast, current genetic approaches to understanding the biology of *F. graminearum* and other fungal plant pathogens are limited to the comparison between individual mutants and a wild type parental strain. This limits the number of meaningful phenotypic assays that can be performed on mutant strains when characterising gene function We have been testing the utility of competitive fitness assays in *F. graminearum* by generating a suite of mutants carrying unique molecular barcodes flanked by common priming sites to allow the quantitative analysis of mutant fitness in pools using amplicon sequencing. Initially a set of 20 mutants was

developed and here results of relative fitness of these mutants in the presence of the plant defence compound benzoxazolinone (BOA) will be presented. Beyond these initial experiments, relative fitness of mutants during infection time courses will be quantitatively analysed to understand the role of individual pathogen genes in the infection process.

238. Completion of the genome sequence of the ascomycete fungus *Fusarium graminearum*. Robert King[1], Martin Urban[2], Michael Hammond-Kosack[2], Keywan Hassani-Pak[1], Kim Hammond-Kosack[2]. 1) Department of Computational and Systems Biology, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; 2) Department of Plant Biology and Crop Science, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK.

Accurate genome assembly and gene model annotation are critical for comparative species and gene functional analyses. Here we present the completed genome sequence and annotation of the reference strain PH-1 of the ascomycete fungus *Fusarium graminearum*, the causal agent of head scab disease of small grain cereals which threatens global food security. Completion was achieved by combining (a) the BROAD Sanger sequenced draft, with (b) the gene predictions done by the Munich Information Services for Protein Sequences (MIPS) V32, with (c) *de novo* whole-genome shotgun re-sequencing using 100 bp reads, (d) re-annotation of the gene models using RNA-seq evidence and Fgenesh, Snap, GeneMark and Augustus prediction algorithms, followed by (e) manual curation. We have comprehensively completed the genomic 36,563,796 bp sequence by replacing unknown bases, placing supercontigs within their correct loci, correcting assembly errors, and inserting new sequences which include for the first time complete AT rich sequences such as centromere sequences, subtelomeric regions and the telomeres. Within the genome 1529 gene models have been modified and 412 new gene models predicted, with a total gene call of 14,164. The re-annotation impacts upon 69 entries held within the Pathogen-Host Interactions database (PHI-base) which stores information on genes for which mutant phenotypes in pathogen-host interactions have been experimentally tested, of which 59 are putative transcription factors, 8 kinases, 1 ATP citrate lyase (ACL1), and 1 syntaxin-like SNARE gene (GzSYN1). This fully completed *F. graminearum* PH-1 genome and manually curated annotation provides the optimum resource to perform interspecies comparative analyses and gene function studies. The completed *F. graminearum* genome is available at Ensembl Fungi.

This work is supported by the BBSRC, through the Institute Strategic Programme 20:20 Wheat®.

239. The added value of –omic approach to dissect phytopathogenicity of *Fusarium graminearum*: from genomic to genetic. Benoit LAURENT[1], Magalie MOINARD[1], Cathy SPATARO[1], Pauline LASSERRE-ZUBER[2], Nadia PONTS[1], Marie FOULONGNE-ORIOL[1]. 1) MycSA, INRA, Villenave D'Ornon, France; 2) GDEC, INRA, Clermont-Ferrand, France.

Fusarium graminearum sensu stricto is one of the main causal agents of the *Fusarium* head blight on many cereals worldwide. The disease increases yields losses while decreasing grains quality, causing important economic losses every year. Moreover, contamination of grains by stables mycotoxins is an important sanitary concern. Current control strategies do not prevent efficiently from toxin contamination and new knowledge is needed. *F. graminearum* is homothallic but has abilities to outcross in fields (Zeller et al, 2004), generating genetic and phenotypic diversity. This work aims at understanding the differences in pathogenicity and mycotoxin accumulation observed among isolates through genetic approaches. We sequenced six strains of *F. graminearum sensu stricto* at ~94X coverage, contrasted for pathogenicity and mycotoxin accumulation on wheat. Alignments of genomic sequences on reference genome (Cuomo et al, 2007) revealed more than two hundred thousand variants, both SNPs and INDELs. We analyzed the genomic distribution of the variants as well as their possible functional subsequent impact, with a focus on genes described as acting in secondary metabolism, pathogenicity and plant-host interactions. Those results present bases for further association genetic studies and call attention to *Fusarium graminearum* ability's to adapt quickly to new environments. We further integrated the latter genomic data in a targeted approach of forward genetics and used SNPs as molecular markers for analyzing a new segregating population from a cross between two of the six sequenced strains. The sequencing of 96 individuals of this population is ongoing and will permit the construction of a high-density genetic map. Association of genetic patterns to several traits linked to pathogenicity and mycotoxin accumulation *in planta* and *in vitro* will help the discovery of genetic elements controlling those traits, their importance and their possible interactions. Such elements will enable the characterization of new targets for innovative strategies to combat this pathogen and avoid contamination of cereals.

240. Functional analysis of a putative transcription factor Fzt1 in *Fusarium graminearum*. Yoonji Lee, Hokyoung Son, Yin-Won Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea.

Fusarium graminearum is an important plant pathogen which causes *Fusarium* head blight (FHB) on cereal crops and ear rot on maize worldwide. Ascospores produced in fruiting bodies (perithecia) are thought to be the primary inocula for FHB epidemics. Perithecium development is a complex cellular differentiation process mediated by diverse signaling pathways. Characterization of transcription factors (TFs) that are specifically involved in perithecium formation would be valuable for understanding of sexual development. In this study, we selected a putative zinc-finger transcription factor, designated Fzt1, which was previously known to be related to perithecial development. The gene encoding Fzt1 contains a fungal $Zn(II)_2Cys_6$ binuclear cluster domain and is conserved in the subphylum Pezizomycotina of Ascomycota. Deletion mutant of *FZT1* showed a defect in sexual development, but not in other biological processes, including vegetative growth, asexual development, and virulence in *F. graminearum*. A mutant constitutively expressing *FZT1* produced increased number of perithecia compared to the wild-type strain, suggesting that this TF regulates the genes involved in perithecium production. Nuclear localization of the Fzt1 protein supported its role as a transcriptional regulator. This study will allow us to link upstream signaling pathways with the previously characterized downstream genes in sexual development of *F. graminearum.* .

241. Genome-wide functional analyses of cytochrome P450 monooxygenases in *Fusarium graminearum*. Jiyoung Shin, Hokyoung Son, Yin-Won Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea.

Fusarium graminearum is a plant pathogen which causes *Fusarium* head blight in cereal crops such as wheat and barley. The fungus also produces mycotoxins (trichothecenes and zearalenone) in the infected grains, posing a health threat to humans and animals. Cytochrome

P450 monooxygenases (CYP_{450}) constitute a large superfamily of monooxygenases widely distributed in organisms. CYP_{450} are involved in a variety of metabolic processes such as the biosynthesis of steroid hormones, membrane sterols and cell wall compounds. In addition, detoxification of xenobiotics is accomplished with various CYP_{450}-dependent reactions. In *F. graminearum*, only four CYP_{450} are known to be required for trichothecene biosynthesis. In order to characterize the functions of *F. graminearum* CYP_{450}, we identified 116 genes encoding CYP_{450} and 91 CYP_{450} genes were successfully deleted. Disruption of genes was confirmed by PCR screening followed by Southern hybridization. The confirmed mutants were analyzed for changes under 17 phenotypic categories and several xenobiotic inductions to build a comprehensive phenotypic dataset (phenome). The phenotypic categories include vegetative growth, sexual and asexual reproduction, virulence, mycotoxin production, and various stress responses. These data will be valuable resources to elucidate biochemical functions of CYP_{450} in filamentous fungi.

242. Intraspecies interaction of *Fusarium graminearum* contributes to reduced toxin production and virulence. Sean Walkowiak[1,2], Christopher Bonner[1,2], Li Wang[1], Barbara Blackwell[1], Rajagopal Subramaniam[1,2]. 1) Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; 2) Carleton University, Ottawa, Ontario, Canada.

Fusarium graminearum is a pathogenic fungus that causes Fusarium Head Blight in wheat and lowers the yield and quality of grains by contamination with the trichothecene mycotoxin, deoxynivalenol. In Canada, *F. graminearum* exists as two main chemotypes: 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol. These chemotypes have reported differences in their distribution/prevalence, growth, and aggressiveness towards wheat. These chemotypes also coexist and interact with other *Fusaria*, as well as other plant pathogenic fungi and bacteria in the field. We performed whole genome sequencing and comparative genomics of an isolate of each chemotype. To understand the potential interactions between isolates of these two chemotypes, co-inoculation studies were conducted both in culture and *in planta*. The studies showed that intraspecies interaction reduce trichothecene yield in culture and disease symptoms in wheat. To elucidate the genes involved in the intraspecies interaction, expression profiling was performed on RNA samples isolated from co-inoculated cultures.

243. A large-scale functional analysis of putative target genes of mating-type loci provides insight into the regulation of sexual development of the cereal pathogen *Fusarium graminearum*. Hee-Kyoung Kim, Seong-Mi Jo, Gi-Yong Kim, Da-Woon Kim, Sung-Hwan Yun. Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam South Korea.

Fusarium graminearum, the causal agent of Fusarium head blight in cereal crops, produces sexual progeny (ascospore) as an important overwintering and dissemination strategy for completing the disease cycle. This homothallic ascomycetous species does not require a partner for sexual mating; instead, it carries two opposite mating-type (*MAT*) loci in a single nucleus to control sexual development. To gain a comprehensive understanding of the regulation of sexual development in *F. graminearum*, we used in-depth and high-throughput analyses to examine the target genes controlled transcriptionally by two-linked *MAT* loci (*MAT1-1, MAT1-2*). We hybridized a genome-wide microarray with total RNAs from *F. graminearum* mutants that lacked each *MAT* locus individually or together, and overexpressed *MAT1-2-1*, as well as their wild-type progenitor, at an early stage of sexual development. A comparison of the gene expression levels revealed a total of 1,245 differentially expressed genes (DEGs) among all of the mutants examined. Among these, genes involved in metabolism, cell wall organization, cellular response to stimuli, cell adhesion, fertilization, development, chromatin silencing, and signal transduction, were significantly enriched. Protein binding microarray analysis revealed the presence of core DNA binding sequences (ATTAAT or ATTGTT) for the HMG (high mobility group)-box motif in the MAT1-2-1 protein. Targeted deletion of 106 DEGs revealed 24 genes that were specifically required for sexual development, most of which were regulated transcriptionally by both the *MAT1-1* and *MAT1-2* loci. Taken together with the expression patterns of key target genes, we propose a regulatory pathway for *MAT*-mediated sexual development, in which both *MAT* loci are activated by several environmental cues via chromatin remodeling and/or signaling pathways, and then control the expression of at least 1,245 target genes during sexual development via regulatory cascades and/or networks involving several downstream transcription factors and an RNA interference pathway.

244. Comparative transcriptomics reveals common regulators of pathogenicity of *Fusarium oxysporum* strains with different host-specificity. Sarah Maria Schmidt, Joanna Lukasiewicz, Martijn Rep. SILS, University of Amsterdam, Amsterdam, 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 many different *formae speciales*. All strains share a common core genome and possess additional lineage-specific (LS) chromosomes. The lineage-specific chromosomes encode effectors - small proteins that the fungus secretes in the host to promote infection. We wished to uncover genes that are required for pathogenicity towards multiple hosts.

In a comparative transcriptomics approach of the tomato pathogen *F. oxysporum* f. sp. *lycopersici* (*Fol*) and the melon pathogen *F. oxysporum* f. sp. *melonis* (*Fom*), infecting their respective hosts melon and tomato, host-specific effector genes are among the most highly expressed genes during plant infection. In addition to lineage-specific genes, we have identified genes that are highly expressed during plant infection in both pathogens. Among these are 24 genes coding for transcription factors. Two of the transcription factors belong to a small gene family of *FTF1* (Fusarium transcription factor 1) homologs. One copy of *FTF1* is present on the core genome, while the others reside in the LS region of *Fol*. Gene deletion of the core *FTF1* copy in *Fol* resulted in reduced virulence of the transformed strain. We are currently producing gene deletions of some of the other putative common regulators as well as a knock-out mutant of the core *FTF1* homolog in *Fom*.

245. Genome dissection of a *Fusarium oxysporum* isolate that causes fusariosis. Yong Zhang, Greg DeIulio, Li Guo, Li-Jun Ma. biochemistry & molecular biology, UMass, Amherst, Amherst, MA.

Advances in medical treatments increase the complexity of patient populations with immunodeficiency disorders. Opportunistic fungi

have emerged as important causes of morbidity and mortality in immunocompromised individuals. Fusariosis, the infection caused by *Fusarium* spp., is the second most common opportunistic infection caused by filamentous fungi, after aspergillosis. *F. oxysporum* causes localized or disseminated infections that may become life-threatening in neutropenic individuals. Some members of the *F. oxysporum* species complex also cause devastating plant wilt diseases. Horizontally transferred supernumerary (SP) chromosomes determine host-specific pathogenicity of plant-infecting *F. oxysporum* isolates; however, it is unknown whether SP chromosomes also contribute to the increasing cases of fusariosis in humans. Here we dissected the genome of the clinical *F. oxysporum* isolate NRRL32931. Optical mapping technology revealed a compartmentalized genome structure with four unique SP chromosomes (1.8, 1.3, 1.2, and 1 Mb in size). Functional annotation of the 812 genes encoded by the SP chromosomes indicates a potential role in survival and proliferation of the pathogen under high temperature, high pH and ion-poor conditions, similar to those encountered in the human body. This study provides the first evidence for the presence of SP chromosomes in a human-infecting fungus, revealing a potential role of genome compartmentalization in rapid adaptation of *F. oxysporum* to the human host and in the establishment of invasive mycoses.

246. Cyclooxygenase homolog deletion in a Dutch elm disease pathogen. Erika Naruzawa[1], Fabienne Malagnac[2,3], Philippe Silar[2,3], Louis Bernier[1]. 1) Centre d'Étude de la forêt, CEF, Université Laval, Quebec, Canada; 2) Université Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 3) Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain, IED, Paris, France.

Ophiostoma species cause Dutch elm disease (DED) which devastated elms in Europe and North America. These fungi feature a dimorphism in which yeast cells spread passively inside the trees' xylem sap and mycelium invades adjacent vessels and spreads laterally within the xylem. This transition might be connected to pathogenicity. *Ophiostoma* dimorphism was reported to be affected by inhibitors of lipoxygenase and cyclooxygenase. These enzymes are known as dioxygenases. Through a bioinformatics search in the KEGG database, a homolog of the *Aspergillus* cyclooxygenase *ppo* gene, but no homolog of lipoxygenase genes, was found in the *O. novo-ulmi* genome. We aimed to delete the cyclooxygenase homolog to verify its implication in *O. novo-ulmi* dimorphism. To do this, *O. novo-ulmi* strains were first disrupted in the NHEJ gene *mus52*. The construction for deletion of the cyclooxygenase gene was produced by cloning PCR fragments of 500pb for both upstream and downstream flanking regions of the gene into a geneticin resistance cassette. Transformation of *Δmus52* yeast like cells with lithium acetate was employed. Mutants for the *ppo* gene were identified as they grew in media amended with geneticin. PCR and Southern Blot analyses confirmed that the cyclooxygenase gene was disrupted. After crossing with a wild type strain and verification by Mendelian genetics, two strains were observed to have the correct deletion. Cyclooxygenase-deleted F1 progeny strains were recovered and characterized phenotypically for growth on solid and liquid media, response to inhibitor of cyclooxygenase, resistance to hydrogen peroxide and virulence. Both strains showed no clear difference of phenotype compared to wild types. These data suggest that cyclooxygenase has no role in dimorphism or virulence of DED pathogens.

247. Genome-wide analysis of the regulations of genes involved in carbon catabolism through expression Quantitative Trait Loci (eQTL) in *Coprinopsis cinerea*. J. Chang, C.H. Au, C.K. Cheng, H.S. Kwan. School of life sciences, The Chinese University of Hong Kong, Hong Kong.

Lignocellulose is the most abundant natural resource and its conversion into renewable energy attracts many research interests. Understanding the regulation of carbohydrate-active enzymes is fundamental to the use of wood-decaying basidiomycetes in lignocellulose conversion. Our goal is to identify eQTLs of lignocellulolytic enzymes in *Coprinopsis cinerea*, of which the genome harbors high number of Auxiliary Activities enzymes.

We sequenced *C. cinerea* reference strain Okayama 7#130 and its mapping partner #172 to develop a panel of SNP markers. Single spore isolates from crosses of the two strains were sequenced. The parental strains and the 46 single spore isolates were cultured on softwood-enriched sawdust to induce lignocellulolytic enzymes. RNAs from these cultures were sequenced. The RNA-seq results were aligned to the reference transcriptome using BWA. To assess the genetic contribution to expression variations among the 46 segregants, we mapped eQTL genome-widely using the linear regression model. SNPs were estimated for their effect size to explain the expression variances. Genes expressed in similar patterns with the lignocellulolytic enzyme genes across the 46 segregants were also clustered and analyzed.

Three cis-eQTLs and 97 trans-eQTLs ($P < 5 \times 10^{-5}$) have been obtained. These eQTLs have provided us with a wealth of information that the expressions of genes turning off the Carbon Catabolite Repression (CCR) are correlated with SNP. We report one transcription factor as trans-eQTL hotspot, which may regulate the expression of laccase3 and laccase5.

The eQTL approach has identified transcriptional regulation that may contribute to the CAZymes expression. The results will be practically important to the enzyme production, which will benefit the bioethanol production from lignocellulose.

248. Uncovering the capabilities of *Agaricus bisporus* to degrade plant polysaccharides throughout its life cycle. A. Patyshakuliyeva[1], H. Post[2,3], M. Zhou[1], E. Jurak[4], A. J. R. Heck[2,3], K. S. Hildén[5], M. A. Kabel[4], M. R. Mäkelä[5], A. F. M. Altelaar[2,3], R. P. de Vries[1]. 1) Fungal Physiology, CBS-KNAW, Utrecht, The Netherlands; 2) Biomolecular Mass Spectrometry and Proteomics, Utrecht University, Utrecht, The Netherlands; 3) Netherlands Proteomics Centre, Utrecht, The Netherlands; 4) Wageningen University, Laboratory of Food Chemistry, Wageningen, The Netherlands; 5) Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

The common edible mushroom *Agaricus bisporus* is a basidiomycete that thrives on decaying plant material in the forests and grasslands of North America and Europe. It is adapted to forest litter and contributes to global carbon recycling, degrading cellulose, hemicellulose and lignin in plant biomass to oligomers and monomers. *A. bisporus* is also an edible mushroom that is widely cultivated and economically important. But the process of growing *A. bisporus* in compost and utilization of this substrate is poorly understood. In this study, we

performed a wide analysis of genes encoding plant biomass degrading enzymes using high throughput sequencing (RNA-Seq) and lignocellulolytic enzymes secretion by *A. bisporus* grown in compost under commercial conditions to understand the carbon nutritive needs of the fungus and its capabilities to degrade plant biomass.Clear correlations were observed between secreted extracellular polysaccharide degrading enzymes, the expression of the corresponding genes and the composition of compost, which is rich in plant material such as cellulose and hemicellulose. Differences in the expression of genes from different stages of development were detected between spawning, pinning and harvesting stages, suggesting that as soon as the monosaccharides that were released during composting are depleted, *A. bisporus* starts producing enzymes to degrade plant biomass components to satisfy its nutritive needs. Interestingly, our results also uncovered differences in gene expression and secreted proteins between two different harvesting flushes that might be corresponded to the loss in number of mushrooms during second harvesting flush.

249. A genomic survey of proteases in Aspergilli. Miaomiao Zhou[1,3], Sebnem Ozturkoglu Budak[1,2,3], Carlo Brouwer[1], Ad Wiebenga[1,3], Isabelle Benoit[1,3], Marcos Di Falco[4], Adrian Tsang[4], Ronald de Vries[1,3]. 1) CBS-KNAW, Utrecht, Netherlands; 2) Faculty of Agriculture, Department of Dairy Technology, University of Ankara, Ankara, Turkey; 3) Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands; 4) Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke Street West, Montreal, QC H4B 1R6 Canada.

Proteases can hydrolyze peptides in aqueous environments. This property has made proteases the most important industrial enzymes by taking up about 60% of the total enzyme market. Microorganisms are the main sources for industrial protease production due to their high yield and a wide range of biochemical properties. Several Aspergilli have the ability to produce a variety of proteases, but no comprehensive comparative study has been carried out on protease productivity in this genus so far.

We have performed a combined analysis of comparative genomics, proteomics and enzymology tests on seven *Aspergillus* species grown on wheat bran and sugar beet pulp. Putative proteases were identified by homology search and Pfam domains. These genes were then clusters based on orthology and extracellular proteases were identified by protein subcellular localization prediction. Proteomics was used to identify the secreted enzymes in the cultures, while protease essays with and without inhibitors were performed to determine the overall protease activity per protease class. All this data was then integrated to compare the protease productivities in Aspergilli.

Genomes of *Aspergillus* species contain a similar proportion of protease encoding genes. According to comparative genomics, proteomics and enzymatic experiments serine proteases make up the largest group in the protease spectrum across the species. In general wheat bran gives higher induction of proteases than sugar beet pulp. Interesting differences of protease activity, extracellular enzyme spectrum composition, protein occurrence and abundance were identified for species. By combining *in silico* and wet-lab experiments, we present the intriguing variety of protease productivity in Aspergilli.

250. Genome Sequences of *Magnaporthe grisea* isolated from a crabgrass. Kyeongchae Cheong[1,5], Jaehyuk Choi[2], Sook-Young Park[1,3], Jongbum Jeon[1,5], Ki-Tae Kim[1,5], Jaeyoung Choi[4], Gir-Won Lee[6], Yong-Hwan Lee[1,4,5,7]. 1) Fungal Bioinformatics Laboratory, Seoul National University, Seoul 151-921, Korea; 2) Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Incheon 406-772, Korea; 3) Korean Lichen Research Institute, Sunchon National University, Sunchon 540-742, Korea; 4) Department of Forest Sciences, University of Helsinki, 00014 Helsinki, Finland; 5) Department of Agricultural Biotechnology, College of Agriculture and Life science, Seoul National University, Seoul 151-921, Korea; 6) National Instrumentation Center for Environmental Management, Seoul National University, Seoul 151-921, Korea; 7) Center for Fungal Pathogenesis, Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, Seoul National University, Seoul 151-921, Korea.

Magnaporthe grisea, a species isolated from crabgrass, is phylogenetically different from *M. oryae* infecting rice and other grass species. *M. oryzae* is the best studied model fungus for studying plant-pathogen interactions and its genome sequence has been publicly available. However, no information is available on genome sequence for *M. grisea*. *M. grisea* isolates favor infecting a crabgrass than the other grass hosts, but they still have ability to develop disease symptoms on rice. Here we report two draft genome sequences of *M. grisea* strain W97-11 and W98-20. Their genomes were sequenced using a high-throughput Illumina sequencing technology and sequencing reads were assembled using a SOAPdenovo. Genome sequences of W97-11 and W98-20 were assembled 47,589,632bp of 113 scaffolds (≥1,000bp) and 37,796,700bp of 258 scaffolds (≥1,000bp), respectively. A total of 12,121 and 12,193 protein-coding sequences of W97-11 and W98-20 were predicted using the MAKER pipeline. Genome sequences of *M. grisea* were further comparatively analyzed with genomes of related fungal species including *M. oryzae* (lab strain 70-15 and field strain KJ201), *M. poae*, and *Gaeumannomyces graminis*. The annotated genomes were clustered by Tribe MCL and a total of 5,768 core clusters were found. This analysis indicates that *M. grisea* is evolutionarily closer to *M. oryzae* rather than the others. These data will provide new insights on host specificity and evolutionary relationship among species of *Magnaporthe*.

251. Comparative analysis of draft genome sequences of the conifer tree pathogen *Heterobasidion annosum s.s.*. Jaeyoung Choi[1], Gir-Won Lee[2], Jongbum Jeon[3], Ki-Tae Kim[3], Nicolas Détry[1], Hsiao-Che Kuo[1], Hui Sun[1], Fred Asiegbu[1], Yong-Hwan Lee[1,3,4]. 1) Department of Forest Sciences, University of Helsinki, 00014 Helsinki, Finland; 2) National Instrumentation Center for Environmental Management, Seoul National University, Seoul 151-921, Korea; 3) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; 4) 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.

The causal agent of root and butt rot of conifer trees, *Heterobasidion annosum*, is widespread in boreal forests and economically responsible for annual loss of approximately 50 million euros to forest industries in Finland alone and much more at European level. In order to further understand the pathobiology of this fungus at the genome level, a Finnish isolate of *H. annosum* sensu stricto (isolate

03012) was sequenced and analyzed with the genome sequences of 24 white-rot and 13 brown-rot fungi. The draft genome assembly of *H. annosum* has a size of 31.01 Mb, containing 11,453 predicted genes. Whole genome alignment showed that 82.32% of *H. annosum* genome sequences were aligned with those of previously sequenced *H. irregulare* TC 32-1 counterparts. The result is further supported by the protein sequence clustering analysis which revealed that the two genomes share 6,719 out of 8,647 clusters. When sequencing reads of *H. annousm* were aligned against the genome sequences of *H. irregulare*, six single nucleotide polymorphisms (SNPs) were found in every 1 kb, on average. In addition, more than 99% of SNPs were found to be homo-variants, suggesting that the two species have long evolved from different niches. Furthermore, 24 white-rot fungi shared 2,564 clusters which contain 5,417 genes, on average. Comparative gene family analysis revealed that most of the white-rot fungi investigated had more gene families involved in lignin degradation or modification, including laccases and peroxidase. Comparative analysis of the two *Heterobasidion* spp. as well as white-/brown-rot fungi provided new insights for understanding the pathobiology of the conifer tree pathogen and their potential role in carbon cycling in nature.

252. 1000 Fungal DNA Samples for the 1000 Fungal Genome Project. David Culley[1], Mark Butcher[1], Kerrie Berry[2], Igor Grigoriev[2], Jon Magnuson[1]. 1) Chem and Biol Process Dev, Pacific Northwest National Labs, Richland, WA; 2) DOE Joint Genome Institute Mountain View, CA.

Fungi are ubiquitous organisms found in many extreme environments filling many ecological niches. These organisms encompass one of the largest and most diverse phylogenetic branches and are a major source of metabolic capabilities important in natural ecosystems. Our understanding of carbon and nutrient cycling, as well as many industrial and pharmaceutical processes benefit from what can be learned from the genomes of fungi. The 1000 Fungal Genome Project (FGP) is an international effort, in collaboration with the DOE Joint Genome Institute, with the goal of generating two genome sequences from each of the more than 500 families of the Fungal Tree of Life. The ultimate goal of this project is to establish a broad reference set useful in comparative genomics, metabolic mining, and characterizing ecological roles played by fungi.

A major challenge in completing genomes for the 1000 FGP has proven to be the difficulty in obtaining sufficient high quality genomic DNA and RNA. This problem is likely related to the very diversity that makes this Kingdom so interesting. Fungi produce a huge variety of cell wall materials, waxy compounds, melanins, pigments, secreted polysaccharides and reactive secondary metabolites. In addition, fungi vary tremendously in growth rates and morphological characteristics in cultures. This growth, metabolic and morphological diversity can lead to challenges in isolating gDNA and RNA that stem from problems obtaining sufficient biomass, the presence of senescing tissue, cell lysis and separation from co-purifying compounds

To address problems associated with supplying gDNA and RNA to the 1000 FGP we are developing a work-flow to maximize success with a diversity of fungi and to improve sample throughput. We will report on the results of our investigations related to establishing growth conditions for biomass production, obtaining metadata on growth characteristics, sample handling, gDNA and RNA isolation protocols and quality control.

253. Evolutionary mechanisms and genome diversity in the Sudden Oak Death pathogen *Phytophthora ramorum*. A. Dale[1], S. Everhart[2], N. Feau[1], B. Dhillon[1], J. Tabima[3], G. Bilodeau[4], C. Brasier[5], N. Grunwald[3], R. Hamelin[1]. 1) University of British Columbia, Vancouver, BC, Canada; 2) University of Nebraska, Lincoln, NE, USA; 3) Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR, USA; 4) Canadian Food Inspection Agency, Ottawa ON, Canada; 5) Forestry Commission, Farnham, Surrey, United Kingdom.

Phytophthora ramorum, a recently emerged oomycete pathogen, gained notoriety in the mid to late 1990s, causing bleeding cankers and mortality on *Quercus agrifolia* in the disease sudden oak death. The pathogen affects over 100 hosts and also causes a lethal stem canker on Japanese larch and European beech. A recent host jump to Japanese larch and subsequent wide-scale mortality in plantations in Europe have prompted questions on the adaptability and evolution of this pathogen. Previous studies have shown four reproductively isolated, clonal lineages of *P. ramorum* that are slowly diversifying through mutations. We hypothesized that alternate mechanisms such as homologous recombination drive evolution in these asexual lineages resulting in gene conversion and gene gain or loss, and these events are driven by genome structure including gene density and transposable element content. To detect genomic signatures of recombination and adaptation, and to characterize inter-lineage differences, we sequenced and compared 107 genomes from the four lineages of *P. ramorum*. We observed loss of heterozygosity in 23 isolates in all four lineages, affecting over 100 scaffolds. In the NA1 lineage, one scaffold, containing a large cluster of genes encoding necrosis-inducing enzymes, 18 putative effectors and several carbohydrate active enzymes, is almost entirely homozygous in all isolates. Eight EU1 isolates are also homozygous in half of the same scaffold. The high frequency of this conversion in two lineages suggests that it could result in an advantageous genotype. Lineage specific DNA dominated by transposon like elements, and also composed of pathogenicity related genes also defines inter-lineage diversity. Results suggest that gene conversion is a prominent driver of evolution in *P. ramorum*. Gene conversion could result in expression of recessive alleles, loss of host-recognized effectors, or in mating type switches which could lead to sexual recombination in populations.

254. Stress-resistance and pathogenicity traits in the genomes of black yeasts. Cene Gostinčar[1], Janja Zajc[2], Metka Lenassi[3], Ana Plemenitaš[3], Nina Gunde - Cimerman[2,4]. 1) National Institute of Biology, Ljubljana, Slovenia; 2) Biotechnical Faculty, University of Ljubljana, Slovenia; 3) Faculty of Medicine, University of Ljubljana, Slovenia; 4) Centre of Excellence CIPKeBiP), Ljubljana, Slovenia.

Black fungi are a group of microorganisms with a characteristic morphology and excellent stress tolerance – some of them are even classified as extremophiles. Several black fungi are able to infect plants and animals (including humans), sometimes with devastating consequences. All of these traits, together with substantial biotechnological potentials of some black fungal species, are the reasons for an increasing interest in this fungal group.

The aim of our research was to sequence and compare the genomes of two black fungi. The extremely halotolerant *Hortaea werneckii* can grow in media almost saturated with NaCl. Although not as halotolerant as *H. werneckii*, the polyextremophilic *Aureobasidium* spp. are tolerant to many other types of stress. Besides this, they are excellent producers of extracellular enzymes and are incredibly phenotypically plastic, all of which is reflected in their ubiquitous occurrence.

Interestingly, despite the good adaptation of these species to stressful, even extreme conditions (or, as has also been suggested, precisely because of it), they are also able to switch habitats. Both fungi, for example, are capable of interaction with humans. *H. werneckii* is the causative agent of the *tinea nigra* skin infection, while strains of *Aureobasidium pullulans* var. *melanogenum*, now reclassified as *A. melanogenum*, have been described as the causes of several different infections in humans.

We will present here the results of comparative genomics between *H. werneckii* and *Aureobasidium* spp. (with references to genomes of other fungi) that can be interpreted in light of the high stress tolerance of these species and discuss whether these same traits could also play a role in their ability to cause infections in humans.

255. Large scale sequencing of Dothideomycetes provides insights into genome evolution and adaptation. Sajeet Haridas[1], Pedro Crous[2], Manfred Binder[2], Joseph Spatafora[3], Igor Grigoriev[1]. 1) DOE Joint Genome Institute, Walnut Creek, CA. USA; 2) CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; 3) Oregon State University, Corvallis, OR. USA.

Dothideomycetes is the largest and most diverse class of ascomycete fungi with 23 orders 110 families, 1300 genera and over 19,000 known species. We present comparative analysis of 70 Dothideomycete genomes including over 50 that are as yet unpublished. This extensive sampling has almost quadrupled the previous study of 18 species and uncovered a 10 fold range of genome sizes. We were able to clarify the phylogenetic positions of several species whose origins were unclear in previous morphological and sequence comparison studies. We analysed selected gene families including proteases, transporters and small secreted proteins and correlate them to the varied lifestyles of these Dothideomycetes.

256. Genome and secretome analysis provide insights into keratin decomposition by novel proteases from the non-pathogenic fungus *Onygena corvina*. Y. Huang[1], P. Busk[1,2], F. Herbst[3], L. Lange[1,2]. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, 2450 Copenhagen SV, Denmark; 2) Barentzymes A/S, A.C. Meyers Vænge 15, 2450 Copenhagen SV, Denmark; 3) Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg East, Denmark.

Poultry processing plants and slaughterhouses produce huge quantities of feathers and hair/bristle wastes annually. So far there is no widely accepted, efficient and commercialized process to break down keratinaceous wastes or bio-sidestream products. *Onygena corvina*, a non-pathogenic fungus, grows specifically on feathers, hooves, horn and hair in nature. In our study, *O. corvina* was shown to secrete proteases with high keratinolytic activity which completely degraded duck feathers. To investigate further the industrially relevant enzymes secreted by *O. corvina*, we sequenced the genome of *O. corvina* and used peptide pattern recognition to identify 75 proteases. Comparative analysis indicated that 18 putative proteases from four protease families (M36, M35, M43 and S8) may be responsible for keratin decomposition. Heterogeneous expression of the predicted proteases showed that one protease has high potential for degradation of pig bristles. Furthermore, we fractionated supernatant from *O. corvina* culture broth and identified active fractions with five novel proteases belonging to three protease families (S8, M28 and M3). Enzyme blends composed of three of these five proteases, one from each family, were sufficient for degradation of keratinaceous materials. The proteases involved in keratin decomposition from *O. corvina* were discovered using three different approaches: 1) genomics, bioinformatics, cloning, expression and characterization; 2) mass spectrometry elucidation of protein composition; and 3) fractionation of culture broth supernatant. Activity testing was additionally performed on artificial as well as real keratinaceous substrates. A blend of novel proteases, such as those we discovered, could possibly be used for degrading keratinaceous wastes and provide proteins, peptides and amino acids as valuable ingredients for animal feed.

257. Experimental and genomic approaches to understanding the reproductive systems of the thermophile Myceliophthora heterothallica and other members of the Chaetomiaceae. M. I. Hutchinson[1], A. J. Powell[2], A. Tsang[3], R. M. Berka[4], N. O'Toole[3], I. V. Grigoriev[5], K. Barry[5], A. Robinson[1], D. O. Natvig[1]. 1) Department of Biology, University of New Mexico, Albuquerque, NM, USA; 2) Sandia National Laboratories, Albuquerque, NM, USA; 3) Concordia University, Centre for Structural and Functional Genomics, Montreal, QC, CA; 4) Novozymes Inc., Davis, CA, USA; 5) DOE Joint Genome Institute, Walnut Creek, CA, USA.

Members of the Chaetomiaceae are among the most reported species in studies of biomass degradation. This family also contains many of the best-known thermophilic fungi, which are of industrial interest for their abilities to produce thermostable carbohydrate active enzymes. Since there has been no genetically tractable model for the Chaetomiaceae, we have characterized sexual reproduction in the thermophile *Myceliophthora heterothallica* with the goal of establishing this organism as a model for the group. Genome sequences from *M. heterothallica* as well as other members of the Chaetomiaceae show that protein-coding genes in the mating regions of heterothallic species are conserved relative to other outcrossing Sordariales, with an important exception: mating-type *a* strains from heterothallic species of Chaetomiaceae possess a partial *mat A-1* gene. The *mat A-1* in mating-type *a* strains is truncated for the alpha box region required for fertility in other Sordariales, although it has a conserved intact downstream reading frame. Among species of Chaetomiaceae with teleomorphic states, heterothallism is rarely reported while homothallism is common. Analysis of genomes of homothallic species demonstrates that they possess a true *mat a-1* gene, but it is not linked to the *mat A-1* region. Among filamentous Ascomycota, this represents one recurring evolutionary pathway for derived homothallism, which requires *mat A* and *mat a* regions in a single haploid genome. Phylogenetic analyses reveal that temperature growth responses are evolutionarily plastic within the family, with some clades possessing both thermophiles and mesophiles. These analyses also highlight taxonomic problems, including paraphyly in several groups.

258. Next-generation genome and transcriptome based methods for the exploration of secondary metabolites from marine fungi. Abhishek Kumar, Frank Kempken. Botanisches Inst, Christian-Albrechts-University, Kiel, Germany.

Fungi of marine origin are a potent group of secondary metabolite producers. We aim for sustainable exploration of marine fungal isolates and their encoding natural products under the EU-funded project marine fungi (www.marinefungi.eu). We have established the genomic sequences from three marine isolates, *Scopulariopsis brevicaulis, Pestalotiopsis* sp. and *Calcarisporium* sp. by the use of different next-generation sequencing methods (Roche 454, Illumina and ion-torrent).

We report on different properties of genome assemblies and annotations for these fungi. Several gene families and superfamilies have been analyzed to explore genetic peculiarities of these species along with repeats and transposable element contents. The assembled genome of *Scopulariopsis brevicaulis* is ~32 Mb in size with N50 equals to 88 kb and 935 contigs containing more than 16,000 genes. During the annotation process, we were able to annotate 9340 genes (57.3 %) while 6958 genes (43.7 %) remained non-annotated in *Scopulariopsis brevicaulis* genome. 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene encoding a hybrid NRPS-PKS were found. Similarly, the genome size for *Pestalotiopsis sp.* is ~46 Mb with N50 equals to 71.9 kb and 4186 contigs containing about 23,500 genes, which is surprisingly high for an ascomycete and caused by partial genome duplication. 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 *Calcariosporium sp.* is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing about 15,500 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. 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 compounds.

259. Genomics, systematics and proteomics of the wood-decomposing white rot Basidiomycota Polypore species *Phlebia radiata*. Jaana Kuuskeri[1], Olli-Pekka Smolander[2], Heikki Salavirta[1], Pia Laine[2], Ilona Oksanen[1], Miia R. Mäkelä[1], Kristiina Hildén[1], Petri Auvinen[2], Markku Varjosalo[3], Lars Paulin[2], Taina Lundell[1]. 1) Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, Fungal Biotechnology Laboratory,; 2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory; 3) Institute of Biotechnology, Proteomics Unit, Viikki Campus, University of Helsinki, Helsinki, FINLAND.

The systematically incoherent genus *Phlebia* consists of white rot species which produce a variety of lignocellulose-degrading CAZymes and oxidoreductases. Due to the capability for efficient conversion of wood, plant biomass and harmful organic compounds, the type species of this genus, *P. radiata*, was selected for genome and transcriptome sequencing. The nuclear and mitochondrial genomes of Finnish wild-type heterokaryotic isolate *P. radiata* FBCC43 were assembled by using PacBio RSII and Roche 454 FLX Titanium sequencing platforms. *P. radiata* mitochondrial genome is the second largest (over 156 kbp) annotated for fungi. Nuclear genome sequencing produced 4.34 Gbp of data with mean read length of 8095 bp. HGAP3 pipeline assembly produced 127 contigs with 37 longest containing more than 90% of the assembly (contig N50 1.984 Mbp), summing up to 40.92 Mbp.

In addition to genome sequencing, our aim was to provide an assessment of protein coding genes and proteins that function in the core metabolism, degradation of plant cell wall lignocelluloses and oxidation of lignin-like and polluting compounds. The fungus was cultivated on spruce wood, and extraction of RNA and proteome were performed weekly for three to five parallel cultures. Illumina RNA-seq was performed for transcribed RNA samples and nano LC-(H)ESI Orbitrap MS analysis for peptide identification. During the first week of cultivation on spruce wood, the lignin-modifying class II peroxidases (several LiP and MnP enzymes), many cellulolytic CAZymes and auxiliary hydrogen peroxide producing enzymes were the most identified proteins, when totally over 300 secreted proteins were identified on single or more peptides for each protein. The results show that *P. radiata* has a functional and versatile wood and plant biomass degrading enzyme machinery.

260. Mushroomics of *Lentinula edodes:* genomics and transcriptomics. Hoi Shan Kwan, Chun Hang Au, Man Chun Wong, Lei Li, Yung Yung Lee, Qianli Huang, Wenyan Nong, Man Kit Cheung, Jinhui Chang, Xuanjin Cheng. Food Research Centre and School of Life Sciences, Chinese University of Hong Kong, Hong Kong.

We aim to understand the complex fruiting body development and evolution of the mushrooms. We carried out genomics and transcriptomics of the Shiitake mushroom *Lentinula edodes.* We sequenced the genome of the *L. edodes* monokaryon L54A. Over 13,000 protein-coding genes were predicted from the 40.2 Mb draft genome. Comparative analyses on genome sequences of basidiomycetes and ascomycetes revealed genes expanded in genomes of mushroom-forming fungi. Five functional categories, General function prediction only [R], Signal transduction mechanisms [T], Posttranslational modification, protein turnover, chaperones [O], Transcription [K] and Carbohydrate transport and metabolism [G], dominate in the expanded families. We examined kinome, ubiquitome, transcription factories and CAZy enzymes. AGC kinase subfamily, F-box and paracaspase domain-containing E3 like proteins are significantly expanded in mushroom-forming genomes. We sequenced the genomes of 20 wild and cultivated *L. edodes* strains to explore their relationships. Their morphologies and cultivation characteristics were investigated. With these data, we could start to link some phenotypes with the genotypes using a Genome-Wide Association Studies, GWAS, approach. We performed RNA-Seq of multiple stages and identified genes differentially expressed. Transcriptome age index (TAI) profile and transcriptome divergence index (TDI) profile showed a molecular hourglass pattern over the developmental stages. Young fruiting body stage is the bottleneck expressing the evolutionarily oldest and most conserved transcriptome We compiled the genome sequences and transcriptome of *L. edodes* and other fungi into an Ensembl-based platform with a battery of genomic tools. Our works have generated rich resources for genomics and transcriptomics of mushrooms. The

era of mushroom "omics" or "mushroomics" has come.

261. PhytoPath, an integrative resource for phytopathogen genomics. Helder Pedro[1], Paul Kersey[1], Kim Hammond-Kosack[2], Uma Maheswary[1], Dan Staines[1], Martin Urban[2], Mark McDowall[1]. 1) Ensembl Genomes, EMBL-EBI, Hinxton, Cambridge, United Kingdom; 2) Rothamsted Research.

Achieving sustainable increases in the yield of crop plants will depend on pesticide research and plant breeding. For this, an understanding of gene function is critical, as it determines the total genetic reservoir available to plant breeders. Integrating resources from Ensembl Genomes and PHI-base, PhytoPath (www.phytopathdb.org) organizes (for fungal, oomycete and bacterial pathogens) genome sequence data, genetic variation (DNA and peptide centric) comparative analyses, and phenotypic data to facilitate rese arch on plant pathogenesis. Molecular data is visualized using the Ensembl software suite to provide a highly functional genome browser and a number of alternative routes for programmatic data access; while linkage from genes to disease progression is provided using literature-curated data from the PHI-base resource.
Currently PhytoPath houses more than 40 plant pathogen genomes, from which more than 1400 genes have been associated with disease phenotypes in PHI-base. A simple but powerful “query builder” style interface allows users to progressively select and combine gene-centric data associated with particular hosts, phenotypes, or molecular characterizations.

262. Transposable elements reshaping genomes and favoring the evolutionary and adaptive potential of fungal phytopathogens. Thierry Rouxel, Jonathan Grandaubert, Marie-Helene Balesdent. INRA-Bioger, Thiverval-Grignon, France.

Transposable Elements (TEs) have been considered for long as “junk” DNA in the genome of complex eukaryotes. However, massive sequencing efforts coupled with phylogenetic analyses suggest TEs can act as genome shapers and be a source of gene innovation and genome plasticity, eventually contributing to genome divergence. Fungi are simple and easy to manipulate eukaryote organisms, for which the ever-growing genome information indicates that many plant-associated fungi have a tendency towards genome size expansion. This increase in genome size is mostly driven by TE expansion that eventually shapes adaptive regions of the genome. Such genome regions host genes involved in niche adaptation and favor accelerated evolutionary dynamics of these genes. Focusing on the *Leptosphaeria maculans-Leptosphaeria biglobosa* species complex of closely related plant pathogenic fungi, we will discuss the link between TE invasion/TE bursts in genomes and (i) speciation, (ii) the rise of two-speed genomes, shaping plastic genome environments, (iii) gene diversification that contributed to adaptation to new hosts, (iv) heterochromatine-based regulation of expression of effector genes, (v) accelerated adaptation to resistance gene pressure in gene-for-gene systems.

263. Genome sequencing and comparative analysis of the biocontrol agent *Trichoderma harzianum sensu stricto* TR274. Andrei Steindorff[1,3], Eliane Noronha[1], Cirano Ulhoa[4], Asaf Salamov[3], Alan Kuo[3], Sajeet Haridas[3], Robert Riley[3], Irina Druzhinina[2], Christian Kubicek[2], Igor Grigoriev[3]. 1) Cell Biology, Brasilia University, Brasilia, DF, Brazil; 2) Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering Vienna University of Technology, Vienna, Austria; 3) DOE Joint Genome Institute, Walnut Creek, CA, USA; 4) Cell Biology, Universidade Federal de Goias, GO, Brazil.

Trichoderma harzianum, the most frequent *Trichoderma* species, is well known for their biotrophic interactions with other fungus (mycoparastism) and plants (endophytism). To gain new insights into these biotrophic mechanism used by *T. harzianum sensu stricto*, we sequenced the isolate TR274 genome using Illumina. The assembly was performed using AllPaths-LG with a maximum coverage of 100x. The assembly resulted in 2282 contigs with a N50 of 37033bp. The genome size generated was 40.8 Mb and the GC content was 47.7%, both similar to other *Trichoderma* genomes (http://genome.jgi-psf.org/programs/fungi/index.jsf). Using the JGI Annotation Pipelinene predicted 13,932 genes with a high transcriptome support. CEGMA tests suggested 100% genome completeness and 97.9% of RNA-SEQ reads mapped to the genome. The phylogenetic analysis using 100 orthologous proteins with all *Trichoderma* genomes sequenced at JGI, corroborates the *Trichoderma* (*T. asperellum* and *T. atroviride*), *Longibrachiatum* (*T. reesei* and *T. longibrachiatum*) and *Pachibasium* (*T. harzianum* and *T. virens*) section established based on the standard marker is rpb2. The comparison between the strains TR274 and CBS 226.95 (already sequenced by JGI) suggests a high genome similarity. The secondary metabolites, CAZymes, transporters and proteases contraction and expansion were analysed through CAFE analysis. The results shows that larger genomes (Pachybasium section) accumulated more gene families “gains” and smaller, more “losses” (Longibrachiatum section). Future analysis will improve the understanding of this complex genus and give some insights about its lifestyle and the interactions with the environment.

264. Structural and gene content variation among strains of the maize anthracnose fungus *Colletotrichum graminicola*. Gabriel E. Rech, José M. Sanz-Martín, Serenella A. Sukno, Michael R. Thon. Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, 37185 Villamayor, Spain.

To better understand patterns of genetic variation in the maize pathogen *Colletotrichum graminicola*, we sequenced the genomes of seven field isolates showing a variable range of virulence to maize and collected from different regions of the world. We analyzed genomic structural variations and patterns of gene gain and loss using genomic sequences obtained from various assembly and gene annotation strategies. We identified sets of unique genes in each isolate, and discovered that they are significantly enriched with genes coding for small secreted proteins (putative effectors), which could represent evolutionary innovations directly involved in host specificity or environment adaptation. Pathogenicity and microscopic assays show that one of the isolates grows endophytically within the host. Four genes coding for enzymes directly involved in the degradation of plant cell walls are affected by genomic structural variations occurring only in the genome of the endophytic isolate, suggesting that the disruption of some of these genes could be responsible for the loss of pathogenicity in this isolate. In addition, five of the 72 genes are upregulated in the reference isolate M1.001 during the necrotrophic phase of infection suggesting their involvement in the pathogenic lifestyle of *C. graminicola*. Genes coding for putative effectors were found at or near breakpoints of genomic structural variations, suggesting this mechanism could be involved in promoting variability of these genes. Overall, genomic variation and patterns of gene gain/loss provide a valuable resource for selecting targets for further functional and

population genetic analyses aimed at identifying genes involved in the development of maize anthracnose.

265. Genomic pathways key to intracellular mycoparasitism in the Rozellomycota C. Alisha Quandt[1], Daniele Corsaro[2], Rolf Michel[3], Nicolas Corradi[4], Timothy James[1]. 1) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA; 2) CHLAREAS Chlamydia Research Association, Nancy, France; 3) Laboratory of Medical Parasitology, Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany; 4) Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, ON, Canada.

The relationship between *Rozella allomycis*, an intracellular mycoparasite of *Allomyces*, and Microsporidia, intracellular parasites of animals, has been hypothesized for several years now. The nuclear and mitochondrial genomes of *Rozella allomycis* lack many of the basic genes for primary metabolism but have not undergone genome compaction to the extent seen in the multiple Microsporidia genomes sequenced. More recently, the discovery of diverse and seemingly ubiquitous cryptic fungi closely related to *R. allomycis*, has led to proposal of a single clade of early diverging fungi, called by various names (*e.g.* Cryptomycota, Rozellomycota, Opisthosporidia). Here, we present results from the genomes of Rozellomycota. We analyzed the transcriptional profile of *R. allomycis* growing endoparasitically in dual culture with its host, *Allomyces*, with analysis of the most highly expressed genes, genes involved in parasitism, and comparison with genes present in Microsporidia and other mycoparasitic fungi. We also analyzed the expression of nucleotide transporters in the *R. allomycis* genome which are known to have been horizontally transferred from chlamydia into the common ancestor of *Rozella* and Microsporidia. Because these genes are hypothesized to be involved in energy and nucleotide theft, we hypothesize they may have facilitated the evolution of intranuclear parasitism observed in newly described Rozellomycota such as the amoeba parasite, *Paramicrosporidium*.

266. System-wide functional analysis platform for pathogenicity genes in the rice blast fungus. Sook-Young Park[1,2], Jaehyuk Choi[2], Jaeyoung Choi[2], Seongbeom Kim[2], Jongbum Jeon[2], Seomun Kwon[2], Dayoung Lee[2], Aram Huh[2], Miho Shin[2], Jeil Hong[2], Kyungyong Jung[2], Jaejin Park[2], Junhyun Jeon[2], Seogchan Kang[3], Yong-Hwan Lee[2]. 1) Korean Lichen Institute, Suncheon, South Korea; 2) Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; 3) D ept. 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 (pGOF1), 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. Over 70% of transformants showed increased transcripts of the genes that are found immediately adjacent to the vector, compared to those of wild type. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes. 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. Our over-expressed transformant library would be a valuable resource for functional characterization and this system may be applicable in other fungi.

267. A new tool enabling fungal phospho-proteomic analysis. Nikhil Ramsubramaniam[1], Feng Tao[2], Shuwei Li[3,4], Mark Marten[1]. 1) Dept Chem. Biochem. Environ. Engr., Univ Maryland Baltimore County (UMBC), Baltimore, MD 21250; 2) Omic Biosystems, 9700 Great Seneca Highway, Room 115, Rockville, MD 20850; 3) Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742; 4) Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850.

We demonstrate a novel approach to quantify the phosphorylation state of fungi using Deuterium isobaric Amine Reactive Tag - DiART. DiART is a peptide-mass-tag for proteomic quantification via liquid chromatography-tandem mass spectrometry (LC-MS/MS). While chemically similar to commercially available mass-tags, DiART shows significantly better analytical sensitivity and accuracy, and costs approximately 75% less. The combination of better performance and dramatically lower cost, enables new proteomic workflows. For example, using DiART-tagged mixtures of two synthetic, phosphorylated peptides, and their non-phosphorylated counterparts, we demonstrate the compatibility of DiART with TiO_2-affinity purification of phosphorylated peptides. Comparison of theoretical vs experimental reporter ion ratios reveals accurate quantification of phosphorylated peptides over a dynamic range of more than 15 fold. Using DiART labelling and TiO_2 enrichment (DiART-TiO_2) with large quantities (8mg) of protein from model fungus *Aspergillus nidulans* cell lysate, we quantified 744 unique phosphopeptides. Overlap of TiO_2-enriched phosphopeptide median and theoretical values demonstrates our ability to accurately quantify phosphoproteins in a complex mixture. We will discuss these data, and our efforts to use DiART to elucidate an important gene regulatory network in *A. nidulans*..

268. Metabolic adaptations of *Phytophthora infestans* to different host environments and growth conditions. Meenakshi S Kagda[1], Carol Davis[2], Howard Judelson[2]. 1) Genetics Genomics and Bioinformatics Program, University of California Riverside, Riverside, CA; 2) Plant Pathology Department, University of California Riverside, Riverside, CA.

Nutrient acquisition and metabolic adaptations are important characteristics in pathogen biology. Information about these in the *Phytophthora infestans*-potato/tomato pathosystem will help us better understand disease progression. Consequently, we have annotated the metabolic genes within the *P. infestans* genome, and followed their expression by RNA-seq and using reporter genes in transformants. Approximately 1300 metabolic genes were identified, several of which show novel features and inheritance by horizontal or

endosymbiotic gene transfer. RNA-seq and microarray studies indicate that many are expressed differentially when *P. infestans* is grown on diverse plant hosts and artificial media. Next, fusions with fluorescent proteins were used to study expression of selected genes *in planta.* Consequently, we identified a metabolic protein that localized specifically within haustoria. To the best of our knowledge, this is the first report of a metabolic protein to have a haustorial localization in oomycetes and supports the use of this structure for nutrient uptake.

269. Autophagy relieves the stress of reactive oxygen species (ROS) triggered by caloric restriction (CR). Thomas Hahn[1,2], Fusheng Tang[1], Helen Benes[2], Richard Segall[3]. 1) Information Science, University of Arkansas at Little Rock (UALR), Little Rock, AR; 2) University of Arkansas Medical Science (UAMS), Little Rock, AR; 3) Arkansas State University, Jonesboro, AR.

We previously observed that CR extends lifespan of wild type yeast cells but shortens the lifespan of mutants missing either Atg15 or Erg6. CR also causes ROS to accumulate in the mutants, suggesting that CR may trigger some responses detrimental to lifespan.

To identify such responses, we compared the expression profile of wild type (WT) and mutants under normal and CR-media. In atg15-delta, we found that mitochondrial respiratory chain, mitochondrial organization, and mitochondrial protein localization are differentially regulated by CR than they are in WT. Since a longevity mechanism of CR is the up-regulation of respiration, the altered responses of mitochondria in atg15-delta may explain the accumulation of ROS in the cytoplasm of atg15-delta in CR.

In erg6-delta, more pathways are differentially regulated by CR than in atg15-delta. Cellular amino acid metabolic process, biosynthetic process, and sterol metabolic process are the top 3 pathways that are differentially modulated by CR compared to WT. Inspection of individual pathways showed that CR up-regulates the generation of NADH and NADPH in WT cells. However, many of these genes cannot be up-regulated in erg6-delta. In WT cells, the elevation of NADH and NADPH may counteract the oxidative stress caused by CR.

Erg6-delta cannot up-regulate the production of NADH/NADPH because of the accumulation of ROS in CR. Atg15 is required for lifespan extension longevity mutants mimicking CR. Thus, we propose that autophagy relieves the ROS-stress induced by CR.

270. The Mycorrhizal Genome Initiative: Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis. A. Kohler[1], L. G. Nagy[3], E. Morin[1], C. Veneault-Fourrey[1], E. Lindquist[2], A. Lipzen[2], A. Kuo[2], I.V. Grigoriev[2], D.S. Hibbett[3], F.M. Martin[1], Mycorrhizal Genome Initiative Consortium. 1) Institut National de la Recherche Agronomique, Unité Mixte de Recherche 1136, Interactions Arbres/Microorganismes, Centre de Nancy, Université de Lorraine, 54280 Champenoux, France; 2) US Department of Energy Joint Genome Institute, Walnut Creek, CA 94598, USA; 3) Clark University, Biology Department, Worcester, MA, 01610, USA.

Mycorrhizal symbioses have arisen repeatedly during fungal evolution and include not only ectomycorrhizal (ECM) associations, but also ericoid mycorrhizae (ERM), which are limited to Ericales and a few fungal clades (e.g., Helotiales, Sebacinales), and orchid mycorrhizae (ORM), which are formed by various lineages of Dikarya. It is not known if these mycorrhizal symbioses share the genomic features found in ECM fungi. Here, we assess whether there are general evolutionary and functional properties of mycorrhizal Basidiomycota and Ascomycota, using comparative analysis of 17 newly sequenced genomes, including twelve ectomycorrhizal, orchid and ericoid species, and five saprotrophs, which we analyzed along with other fungal genomes.

Ectomycorrhizal fungi have a reduced complement of genes encoding plant cell wall degrading enzymes (PCWDE), compared to their ancestral wood decayers. Nevertheless, each has retained a unique array of PCWDE, suggesting they possess diverse abilities to decompose lignocellulose.

Similar functional categories of non-orthologous genes are induced in symbiosis. Seven to 38% of induced genes are "orphan" genes, including genes that encode secreted effector-like proteins.

Convergent evolution of the mycorrhizal habit in Fungi occurred via the repeated evolution of a ‘symbiosis toolkit’, with reduced numbers of PCWDE and lineage-specific suites of mycorrhiza-induced genes.

271. Evolution and Diversity of Sex-Related Gene Homologues in a Supposed Asexual Arbuscular Mycorrhizal Fungus. Timea Marton, Philippe Charron, Manuela Kruger, Nicolas Corradi. Department of Biology, University of Ottawa, Ottawa, Ontario, Canada.

Arbuscular mycorrhizal fungi (AMF) are ancient organisms that form symbioses with more than 80% of land plants. Fossil evidence of this partnership dates back 500Ma, when land was first colonized by plants. The mutualistic relationship between host roots and the fungus consists of an exchange of carbohydrates for water and minerals (phosphorus and nitrogen), compounds that are respectively essential to the proliferation of both organisms. Despite their extraordinary longevity, a lack of evidence supporting sexual reproduction has led to assume that AMF are purely clonal organisms. However, recent genome analyses are starting to challenge this notion. Specifically, AMF genomes encode for a large number of homologues of proteins that are linked to sexual processes in other eukaryotes, including several typically involved in partner recognition, such as mating-type high mobility group (MATA-HMG) proteins found in mating-type loci, and others associated with the process of meiosis (meiosis-specific genes). The present study expands the current knowledge of sex-related genes in AMF by exploring new genome data obtained from several isolates of the AMF model *Rhizophagus irregularis*. These new investigations reveal that a single isolate can potentially code 225 MATA-HMGs, or 54% more than previously reported. Furthermore, these genes differ considerably in both number and structure among isolates of *R. irregularis*, supporting the presence of substantial

genome plasticity in this species. Some MATA-HMGs display unique genomic organizations that are reminiscent of operons, whereas others are similar to MAT-loci of the Zygomycota and the Ascomycota phyla. The expression of MAT-loci and meiosis-specific genes has also been studied to provide preliminary insight into their potential function. In retrospect, this research uncovers an unprecedented amount of AMF genes that are homologues to sex-related genes of other fungi, and reveals for the first time their atypical genomic architecture and expression.

272. Structural variation in regions of suppressed recombination associated with Spore killer in *Neurospora intermedia* and *Neurospora sitophila*. Jesper Svedberg[1], Thomas M. Hammond[2], Hanna Johannesson[1]. 1) Department of Organismal Biology, Uppsala University, Uppsala, Sweden; 2) School of Biological Sciences, Illinois State University, Normal, IL.

Spore killer is a meiotic drive element found in natural populations of *Neurospora sitophila* and *Neurospora intermedia*. In a cross between a strain carrying the Spore killer genetic element and strain that is sensitive to Spore killer, half of the spores will die and the surviving spores will all show the killing phenotype in further crosses. The Spore killer element has been mapped to an approximately 2 Mbp large region of suppressed recombination on linkage group 3.

We have sequenced seven genomes which are representative of all known Spore killer types as well as sensitive and resistant strains of *N. sitophila* and *N. intermedia*, using Illumina MiSeq (to 100x coverage) and Pac Bio (to 50x coverage) technologies to create high quality, full chromosome assemblies. This data has revealed a complex pattern of inversions, insertions and deletions that may play an essential role in maintaining the region of suppressed recombination and in explaining the genetic divergence that lies behind the Spore killer phenotype.

We here present a comparative analysis of the genomic architecture in the regions of suppressed recombination among the sequenced strains and what these patterns may mean for the evolution of the Spore killer phenomenon.

273. Prediction of gene functions using phenomics in the model filamentous fungus, *Neurospora crassa*. Ilva Cabrera, Jason Stajich, Katherine Borkovich. Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

Neurospora crassa grows by polar extension of tube-like structures called hyphae. *Neurospora* has a complex life cycle, with two asexual sporulation pathways and a sexual cycle that produces meiotic progeny. In this study we have used phenotypic methods to analyze transcription factor mutants. We have coupled this data with previously published work on serine/threonine protein kinases, in order to analyze specific groupings tied to mitogen activated protein kinase (MAPK) signaling pathways. Quantitative and qualitative features for the transcription factors and kinases were grouped and analyzed using multivariate analyses. Multivariate analyses approaches include Principal Components Analysis (PCA), K means, and unsupervised Random Forests. These analyses revealed gene groupings of conserved MAPK signaling pathways (MAK-1/MAK-2, and OS).

Findings were supported with molecular techniques examining the basal and induced levels of the terminal MAPK. These multivariate analyses confirmed pathways, as well as revealed putative functions for previous unknown genes. This study demonstrated that phenotypic features, qualitative and quantitative, can be utilized to predict function to the many uncharacterized genes in the *Neurospora* genome.

In order to add another phenotypic parameter, we have developed novel algorithms to analyze the formation of a colony, from asexual spore to mature hyphae. We are the first to analyze the asexual spore (conidia) size, hyphal compartment size, and hyphal growth rate in an automated manner. This novel approach employs phenotypic parameters that can be utilized for streamlined analysis of thousands of mutants. This software, to be made publicly available in the future, eliminates subjectivity, allows high-throughput analysis, and saves time in processing.

274. Whole genome sequence analysis to characterize anonymous classical mutants of *Neurospora crassa*. Kevin McCluskey[1], Blake Simmons[2,3], Scott Baker[2,4]. 1) Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, KS; 2) US DOE Joint Bioenergy Institute; 3) US DOE Sandia National Laboratory; 4) US DOE Pacific Northwest National Laboratory.

Strains of *Neurospora crassa* were subject to whole genome sequence analysis to identify and characterize the anonymous mutated DNA sequence associated with their classical genetic locus and its phenotype. Among the classic mutant strains, phenotypic characteristics formed natural groups including auxotrophic and non-utilization mutants, mitochondrial mutants, morphological mutants, and temperature sensitive lethal mutants. Additional strains were sequenced to identify the origins of shared sequence polymorphisms, characterize the impact of repeated strain passage, and of the spontaneous meiotic mutation rate, to reveal the nature and source of second-site mutations in gene deletion mutants, and to characterize chromosome rearrangements including those associated with inseparable phenotypes. 535 DNA miniprep samples were sent for sequence analysis and most have completed resequencing at the time of writing.

275. Sequencing and comparative genomic analyses of divergent *Aspergillus flavus*. B. Adhikari, P. Cotty. USDA-ARS/University of Arizona, Tucson, AZ 85721.

Aspergillus flavus, the fungal species responsible for aflatoxin contamination of many crops, varies widely in distribution, genomic features and ability to produce aflatoxins. *A. flavus* has a vegetative incompatibility system that limits gene flow among the diverse lineages that make up this taxon. Certain *A. flavus* genotypes produce very large concentrations of aflatoxins while others produce no aflatoxin (called atoxigenics). Several atoxigenic genotypes have been developed into biopesticides for the prevention of contamination. To better understand the genomic bases of atoxigenicity and develop genomic resources for both aflatoxin-producing and atoxigenic *A. flavus*, we have sequenced the genomes of five *A. flavus* genotypes with and without aflatoxin-producing ability. Genomes were Illumina sequenced to at least 50X using both short and long-insert libraries. Genome sequences were *de novo* assembled, annotated, and contrasted in order to

identify structural as well as functional differences among gene clusters, expansion and contraction of gene families, and large-size structural variations. Comparative genomic analyses revealed widespread presence of large indels in *A. flavus* genomes. Deletions up to 250 kb unique to specific genotypes were identified. Such regions may serve as diagnostic markers for identification of specific *A. flavus* lineages within fungal populations.

276. First evidence of *Helitrons* transposable elements insertion in fungi. A. Borgognone, A.G. Pisabarro, L. Ramirez. Department of Agrarian Production, Public University Navarre, Pamplona, Spain.

Helitrons are a superfamily of DNA transposons containing specific hallmarks, discovered by computational analysis of eukaryotic genomes. The presence of these elements was recently uncovered by computational and experimental analyses in *Pleurotus ostreatus*, a lignin-degrading basidiomycete of increasing interest for different industrial applications. Previous studies (1) based on sequence analyses showed that helitrons can capture, amplify and express captured host genes, playing an important role in the evolution of many eukaryotes genomes. Putative autonomous helitrons carry a motif similar to the replication initiator (Rep) of plasmid rolling circle replicons, as well as a DNA helicase (Hel) domain. According to the proposed transposition method, these elements belong to class II transposable elements and mobilize through a rolling circle replication (RC) instead of the cut-and paste mechanism used by other class II transposons. Because their novelty, these elements have been poorly studied due to the lack of a system to detect their transposition. In fact, no evidence of a helitron mobilization has ever been shown under laboratory conditions. Here we describe the first evidence of a helitron somatic transposition in the chromosome I of the *P. ostreatus* monokaryotic strain PC15 obtained after de-dikaryotization of the parental strain N001. Results obtained by PCR analysis, Southern blot hybridisation and further confirmed by sequencing analysis of the genome DNA of the strain PC15 showed that the helitron insertion lacked target site duplication. PCR experiments carried out in the genome DNA of the strain N001 and in its progeny were not able to detect any helitron in chromosome I. Taken together, these results suggest the influence of replicative factors on transposition events, leading to rearrangements of the fungal genome and changes in gene expression profiles.

(1) R.Castanera, G.Pérez, L.López, R.Sancho, F.Santoyo *et al*.: **Highly expressed captured genes and cross-kingdom domains present in Helitrons create novel diversity in *Pleurotus ostreatus* and other fungi**. *BMC Genomics* 2014, **15**:1071.

277. Transposable element expansions in *Pleurotus ostreatus* and other fungi. R. Castanera[1], A.G Pisabarro[1], J. Stajich[2], L. Ramírez[1]. 1) Public University of Navarre, Pamplona, Spain; 2) Department of Plant Pathology & Microbiology.University of California, Riverside, California, USA.

Transposable elements are exceptional contributors to eukaryotic genome diversity. Their ubiquitous presence impacts nearly all species by causing mutations that disrupt genes, mediating chromosomal rearrangements and modulating gene expression. Due to the small size of fungal genomes, detection and comparative genomics of repeated sequences is more addressable than in plants or animals. We have developed a bioinformatics pipeline for TE annotation in fungi, based on a set of Python scripts for merging and mining the results of *de novo* and homology-based searches of TEs in assembled genomes. We focused on the comparative analysis between strains of the same species and between species of the same genera (a total of 17 genomes), to investigate the basis of the differences in TE content. The results obtained uncovered large variations between fungal species (from 0.1% to 43.3 % of the genome comprised of mobile elements). A detailed analysis was performed on two strains of *Pleurotus ostreatus* demonstrating high variability in TE content (4.9% in PC9 strain *vs.* 9.9% in PC15 strain). This variability could be attributed to the differential expansion of Class I elements, primarily LTR retrotransposons in the *Gypsy* and *Copia* families. The analysis showed that the PC15 genome contains many more putatively active families of class I, while levels of Class II DNA elements are quite similar. The more active amplification of TEs in PC15 genome compared to PC9 generated a higher number of genes carrying mutations. The PC15 genome contains 41 interrupted genes that were intact in PC9, while PC9 has 9 mutated genes that were intact in PC15. In this sense, the average percentage of genes carrying TE fragments in *P. ostreatus* was 0.8%. Analyses of Repeat Induced Mutation patterns failed to uncover significant differences between both strains, and studies of TE methylation are ongoing. Our results suggest that the equilibrium between host genome defense and TE success critically regulates the expansion of TEs in fungi, and thus their impact on genome architecture and functionality.

278. Intron gains through genomic invasion of Intron-Like Elements in fungi. Ate van der Burgt[1,2], Pierre J.G.M. de Wit[1], Jérôme Collemare[1,3]. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 2) Present address: Dyadic, Wageningen, The Netherlands; 3) Present address: EcoFun team, IRHS-INRA, Beaucouzé, France.

Intron-Like Elements (ILEs) are invasive spiceosomal introns that were identified in six fungal species, where they represent the vast majority of recent intron gains [1]. ILEs differ from Regular Spliceosomal Introns (RSIs) by their longer length and higher stability. Yet, they rapidly degenerate in length and sequence to become undistinguishable from RSIs. It was hypothesized that ILEs are the major mechanism of intron gains in fungi [2]. However, this hypothesis is not supported by their restricted taxonomic distribution. Here, we report the identification of about 10,000 typical ILEs in 53 fungal species that belong to distant classes. Intron gain analyses showed that 96% of inspected ILEs are gained introns, confirming our previous results. Remarkably, we found evidence that new ILEs can originate from other ILEs through sequence insertion, deletion and mutations. Especially in the class of *Dothideomycetes*, long ILEs originate from conserved short elements. Sequence analysis of these short elements revealed the presence of a motif that might be responsible for their ability to multipy because it is more conserved than splicing signals. However, while splicing signals are constrained, this conserved motif rapidly degenerates to become unidentifiable. This conserved motif could also be identified in most of long ILEs. However, in *Sordariomycetes* and *Leotiomycetes*, the motif is slightly different, which may indicate independent origins of ILEs. Altogether, our results show that ILEs are widespread in fungi and regularly emerge to give bursts of intron gains.

1. van der Burgt A, Severing E, de Wit PJ, Collemare J. 2012. Curr Biol. 22(13):1260-5.

2. Collemare J, van der Burgt A, de Wit PJ. 2013. Commun Integr Biol. 6(2):e23147.

279. Preliminary characterization of biofuel relevant phenotypes in a natural population of *Kluyveromyces marxianus* for genome wide association study. Marie K. Donnelly[1,2], Jasmine Yu[2], Jacob Baker[2], Thomas D. Bruns[1,2], John W. Taylor[1,2]. 1) Plant and Microbial Biology, University of California Berkeley, Berkeley, CA; 2) Energy Biosciences Institute, University of California Berkeley, Berkeley, CA.

A chief technical hurdle to industrial cellulosic biofuel production is *Saccharomyces cerevisiae's* sensitivity to high temperatures and toxic products of biomass pretreatment and hydrolysis in the fermentation. While much work has been done to engineer suitable industrial strains, a complementary approach is to look for desirable traits in closely related yeasts, such as the thermotolerant yeast, *Kluyveromyces marxianus*. Few strains of *K. marxianus* exist in culture collections, and the extent of natural variation in traits like thermo-tolerance and pH sensitivity is unknown. To sample the natural variation of *K. marxianus*, we traveled to the sugarcane growing and refining region of Florida and collected sugarcane bagasse from active compost piles where the temperature was between 40 and 50°C. 120 bulk bagasse samples were collected from four geographically distant sites. Bagasse samples were blended with water to create slurries, diluted by 10X and 100X, plated on YM agar at neutral pH and acidic pH conditions, and incubated at 45°C until cultures grew. Cultures were identified by BLAST matches of the amplified ITS region. Cultures identified as *K. marxianus* were isolated and grown up in liquid media to assess phenotypic variation within this population. Genomic DNA was extracted from each individual and sequenced via Illumina HiSeq. Preliminary results show variation within the population in growth above 45°C. In addition to thermo-tolerance, we will investigate variation in response to inhibitors in the growth medium, anaerobic tolerance to ethanol, and utilization of carbon. The goal of this work is to match the phenotypic variation in this natural population with genetic variation using the technique of genome wide association.

280. Whole-Genus Sequencing: 300 Aspergilli. Alan Kuo[1], Robert Riley[1], Alicia Clum[1], Asaf Salamov[1], Scott Baker[2], Blake Simmons[2], Mikael Andersen[3], Igor Grigoriev[1]. 1) Fungal Genomics Program, DOE Joint Genome Institute, Walnut Creek, CA; 2) Joint BioEnergy Institute, Emeryville, CA; 3) Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark.

Aspergillus is a ubiquitous and phenotypically diverse genus of filamentous Ascomycota, many of which play key roles as fermenters in food production, platforms for biotechnology and industrial production of enzymes and chemicals, plant and opportunistic animal pathogens, and agents of agricultural toxigenesis and biomass conversion for bioenergy. As part of a DOE Joint BioEnergy Institute initiative to characterize the entire genus, the JGI plans to sequence, assemble, and annotate the genomes of each of the ~300 species of the genus *Aspergillus*. To accomplish this massive task in a timely manner without sacrificing quality, we have sought to streamline our existing processes as well as explore alternative technologies, especially assembly and annotation of long PacBio sequencing reads. Over the past year we have released on MycoCosm the genomes of an additional 24 *Aspergillus* sp. with preliminary analyses of their phylogenies, secretomes, and secondary metabolism. The next tranche of 92 species is expected soon.

281. Comparative genomics of *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome of bats, and related *Pseudogymnoascus* species. Jonathan Palmer[1], Kevin Drees[2], Jeffrey Foster[2], Daniel Lindner[1]. 1) Center for Forest Mycology Research, USDA Forest Service, Madison, WI; 2) Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH.

White-nose syndrome (WNS) of hibernating bats, caused by the cold-adapted fungus *Pseudogymnoascus destructans*, has spread rapidly in North America and is estimated to have contributed to the death of more than 6 million bats since its introduction in 2006. *Pseudogymnoascus destructans* is a member of the under-studied Pseudeurotiaceae and thus little is known about the biology of this pathogen or its close relatives. Here we sequenced, assembled, and annotated the genomes of *P. destructans* as well as six closely related *Pseudogymnoascus* species not known to be pathogenic. Our analysis identified 4,390 orthologous protein groups from the seven *Pseudogymnoascus* genomes, indicating approximately half of the protein-coding genes are shared among these members of the Pseudeurotiaceae. *Pseudogymnoascus destructans* contains 1,365 unique proteins absent from all of the non-pathogenic species and conversely, there are 843 proteins absent in *P. destructans* that are conserved in the other six genomes. Whole proteome comparisons suggested that the genome of *P. destructans* contains only 50% of the carbohydrate utilizing enzymes (CAZYmes) present in the non-pathogenic *Pseudogymnoascus* species and also has fewer secondary metabolite gene clusters. These data support recent findings of reduced saprophytic growth of *P. destructans* and suggest a long evolutionary history of pathogenicity. An interesting and distinguishing characteristic of the *P. destructans* genome is the expansion of repetitive/transposable elements, which account for as much as 35% of the genome. In contrast, the non-pathogenic species studied here were estimated to contain from 4-18% repetitive/transposable elements. We validated our comparative genomics findings by testing the ability of these fungi to utilize over 190 carbon sources and by investigating the patterns of transposable elements in multiple isolates of *P. destructans*.

282. Phylogenic analysis revealed an expanded C_2H_2-Homeobox subfamily and characterization of C_2H_2 zinc finger gene family in smoke tree wilt fungus *Verticillium dahliae*. Dianguang Xiong, Yonglin Wang, Chengming Tian. College of Forestry, Beijing Forestry University, Beijing, China.

C_2H_2 zinc finger (CZF) proteins are a major class of transcription factors that play crucial roles in fungal growth, development, various stress responses, and virulence. Little genome-wide data is available regarding the roles of CZF proteins in *Verticillium dahliae*, a destructive pathogen that causes vascular wilt disease in more than 200 plant species. We identified a total of 79 typical CZF genes in *V. dahliae*. Comparative analysis revealed that four plant pathogenic fungi, *V. dahliae*, *Fusarium oxysporum*, *Magnaporthe oryzae*, and *Botrytis cinerea*, have comparable numbers of predicted CZF genes with similar characteristics. Phylogenetic analysis identified a C_2H_2-homeobox subfamily in *V. dahliae* containing seven genes with similar gene structures. *V. dahliae* and *F. oxysporum* (Hypocreomycetidae) have more genes of this subfamily than *M. oryzae* (Sordariomycetidae) and *B. cinere*a (Leotiomycetes). Furthermore, gene-expression analysis of the smoke tree wilt fungus *V. dahliae* strain XS11 using digital gene-expression profiling and RT-qPCR revealed that number of

CZF genes were differentially expressed during microsclerotia formation, nutritional starvation, and simulated *in planta* conditions. Furthermore, the expression profiles revealed that some CZF genes were overrepresented during multiple stages, indicating that they might play diverse roles. Deletion of VDAG_03208 significantly block microsclerotia production, and result in severely reduced virulence in smoke trees. Our results provide useful information concerning the functions of CZF genes in microsclerotia formation, nutritional stress responses, and pathogenicity in *V. dahliae*, and form a basis for future functional studies of these genes.

283. Mating-type genes in cereal rust fungi. Guus Bakkeren[1], John Fellers[2], Rob Linning[1], Les Szabo[3], Scot Hulbert[4], Xianming Chen[4,5], Brent McCallum[6], Xiben Wang[6], Richard Hamelin[7], Barry Saville[8], Christina Cuomo[9]. 1) Pacific Agri-Food Res Ctr, Agriculture & Agri-Food Canada, Summerland, BC, Canada; 2) USDA-ARS, Kansas State U., Manhattan, KS, USA; 3) USDA-ARS, Cereal Disease Laboratory, U. of Minnesota, St Paul, MN, USA; 4) Washington State U., Pullman, WA, USA; 5) USDA-ARS, Wheat Genet., Qual., Physiol. & Dis. Res. Unit, Pullman, WA, USA; 6) Agriculture & Agri-Food Canada, Morden, MB, Canada; 7) Forestry Dept., U. of British Columbia, BC & Natural Resources Canada - Québec, QC, Canada; 8) Trent U., Peterborough, ON, Canada; 9) Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Rust fungi have complex life cycles and many complete their sexual and asexual stages on different host plants. Because of their biotrophic life style, ephemeral, enigmatic sexual stage, and recalcitrance to molecular manipulation, nearly no data exists on their mating type systems: cereal rust fungi may display bipolar mating behavior. Using generated genome sequences of the wheat-infecting rusts *Puccinia graminis tritici* (stem rust), *P. striiformis tritici* (stripe rust) and several *P. triticina* (*Pt*, leaf rust) isolates, we shed light on structural features of their mating-type genes. Two divergently transcribed homeodomain (HD)-containing gene pairs are found in each dikaryotic isolate, similar to the paradigm established for *Ustilago* species. Functional characterization of these *Pt* genes shows that their expression can switch heterologous *Ustilago* cells to filamentous growth, indicative of productive mating interactions. Three related pheromone receptor (*Pra*) genes are found in each dikaryotic cell, supporting the hypothesis that tri-allelic recognition systems may be ancestral in basidiomycetes. In the fragmented genomes, no clear linkage between the HD and *Pra* genes is found; in 30 Canadian *Pt* field isolate genomes, 6 HD allele pairs are distinguished, indicating multiple mating types exist and possibly a *pseudo-bipolar* system encompassing loose linkage as has been described for some Microbotryomycetes. Finally, RNAseq data comparing the sexual stages represented by aecio- and pycniospores produced on the alternate host to wheat infection reveal stage-specific transcripts, including effectors.

284. The Chytrid secretome –a comparative analysis of the secretome of an aerobic, anaerobic and pathogenic Chytrid species. L. Lange[1], B. Pilgaard[1], A. Pedersen[2], F. Gleason[3], P. Busk[1]. 1) Aalborg University, Copenhagen Sv, Denmark; 2) Technical University of Denmark, Kongens Lyngby, Denmark; 3) University of Sydney, NSW, Australia.

This study focuses on the fungal secretome and builds on recent developments in genome sequencing and resolution of the phylogeny of the major fungal groupings. The secretome is biologically important as it reflects interaction; not just what the fungus is but how it grows and competes. Chytridiomycota is considered the earliest diverging fungal lineage of free living fungal species as the physiology of the earlier endoparasitic Cryptomycota has a life form where interaction is not well developed. Therefore Chytridiomycota are chosen to elucidate the composition of the most basal fungal secretome. The focus of the present study is a genome sequencing of the aerobic lignocellulose degrading chytrid, *Rhizophlyctis rosea.* The study includes mining of the genome for genes of secreted proteins; recombinant expression and characterization of a selected key enzyme, the GH45 cellulase; a description of the enzyme activity profile of the *R. rosea* secretome; and a characterization of the ecology and substrate association of the *R. rosea* isolate. The study further includes a comparison of the secretome composition of three very different chytrids: *R. rosea*, the sequenced anaerobic rumen chytrid *Orpinomyces* sp (Neocallimastigomycetes) and *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in amphibians. Highly significant differences are observed. The genomes compared are searched using a sequence analysis methodology (Peptide Pattern Recognition) developed by us (group of first author), which allows for alignment-free gene discovery and robust prediction of enzyme function from sequence. The current study includes analysis of how key substrate-degrading secretome enzymes (cellulases, hemicellulases, amylases, proteases) fit in phylogenetic trees, in an attempt to shed light on how these enzymes of the chytrid secretome have developed in perspective of similar developments in other fungal groups. The resulting phylogenetic trees are used as the basis for discussing the possible roles of mechanisms such as horizontal transfer, gene duplication and loss and convergent evolution.

285. How type material improves the quality of genome data. B. Robbertse, C. Schoch. National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Department of Health and Human Services, 45 Center Drive, Bethesda, MD 20892.

Type material ties formal biological names to physical specimens. When a strain or specimen voucher can be clearly associated with a name and genetic data it serves as a crucial reference point for genetic comparisons. Recently curators at NCBI have started to collect and annotate type material data, when available, for all formally described species. This has been applied to a range of records, from single nucleotides to full genomes. The most immediate impact has been in improving the accurate identification of genome data from bacterial strains. By combining type material information with alignment free k-mer trees, wrongly identified bacterial genomes are currently detected with high confidence and corrected in public records. The increase in fungal genomes with type information will make an expansion of this approach feasible in the near future. Here we present details on this and other approaches to improve and access the accuracy of species names for fungal genomes.

286. Endophyte infection of ryegrass alters metabolism, development and response to stress. Pierre-Yves Dupont[1,2], Carla Eaton[1,2], Jason Wargent[3], Susanne Fectner[1], Peter Solomon[4], Jan Schimd[1], Robert Day[5], Barry Scott[1,2], Murray Cox[1,2]. 1) Inst. of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 2) Bio-Protection Research Centre, Massey University, Palmerston North, New Zealand; 3) Institute of Agriculture and Environment, Massey University Palmerston North, New Zealand; 4) Research School of

Biology, College of Medicine, Biology and Environment, Australian National University, Canberra, Australia; 5) School of Medical Sciences, University of Otago, Dunedin, New Zealand.

Beneficial associations between plants and microbes play an important role in both natural and agricultural ecosystems. Associations between *Epichloë* endophytes and cool season grasses are well-known for their ability to increase resistance to insect pests, fungal pathogens and drought. However, little is known about the molecular changes induced by endophyte infection. Here we show that infection causes dramatic changes in expression of over one third of the host genes. This is in stark contrast to mycorrhizal associations, where substantially fewer changes in host gene expression are observed, and is more similar to pathogenic interactions. We reveal that endophyte infection triggers reprogramming of host metabolism, favoring secondary metabolism at a cost to primary metabolism. Infection also induces changes in host development, particularly trichome formation and cell wall biogenesis. Importantly, this work sheds light on the mechanisms underlying the enhanced resistance to drought and superinfection by fungal pathogens provided by endophyte infection. Importantly, our study reveals that not all beneficial plant-microbe associations behave the same in terms of their effects on the host.

287. Adaptive evolution of fungal phytopathogens - Infection patterns in compatible and non-compatible host-pathogen interactions. Janine Haueisen[1,2], Eva Stukenbrock[1,2]. 1) Environmental Genomics, Christian-Albrechts University, Kiel, Germany; 2) Environmental Genomics, Max Planck Institute for Evolutionary Biology, Plön, Germany.

Phytopathogenic fungi coevolve with their hosts and adapt to their host as an ecological niche. The ascomycete *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*) is a worldwide distributed leaf pathogen of wheat. In contrast, its closest relatives *Z. pseudotritici* and *ardabiliae* are endemic to the Middle East. They occur in natural grasslands and infect leaves of wild grasses. This complex of *Zymoseptoria* species provides us a unique model system to study adaptive evolution within and among closely related hemibiotrophic pathogens. These fungi share a common spatial origin but are adapted to different hosts and ecosystems. Moreover, previously conducted whole genome coalescence analyses suggest that speciation of *Z. tritici* coincides with the domestication of wheat 11,000 years ago. Our goal is to assess how adaptive evolution has shaped the infection process of the three *Zymoseptoria* species during divergence and specialisation to distinct hosts and environments. We use confocal laser scanning microscopy to study how three *Z. tritici* isolates develop in their compatible host (wheat) and have identified and characterised four common infection stages: A - Infection establishment, B - Biotrophic growth, C - Lifestyle transition and D - Necrotrophic growth and asexual reproduction. For isolates of *Z. pseudotritici* and *ardabiliae* we could only find characters of the initial stage and thereby consider their interactions with wheat as incompatible. Following an isolate-specific sampling schedule we have collected total RNA of infected wheat leaves and sequenced the transcriptomes for three compatible and two incompatible interactions at all defined infection stages. Thus, we have 28 host-pathogen RNAseq datasets. We aim to relate the descriptive microscopy data to the respective fungal expression profiles to conduct a new type of comparative analysis. This unique combination of microscopy and large-scale transcriptome analysis will provide novel insights to the phenotypic and functional differentiation in the *Zymoseptoria* species complex and serve as model for studies of other pathosystems.

288. Genome-wide Transcriptional Profiling during Rice-*Magnaporthe oryzae* Interactions. Gir-Won Lee[1], Sook-Young Park[2], Jongbum Jeon[3], Jaeyoung Choi[4], Ki-Tae Kim[3], Seongbeom Kim[3], Yong-Hwan Lee[3,5]. 1) National Instrumentation Center for Environmental Management, Seoul National University, Seoul 151-921, Korea; 2) Korean Lichen Research Institute, Sunchon National University, Sunchon 540-742, Korea; 3) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; 4) Department of Forest Sciences, University of Helsinki, 00014 Helsinki, Finland; 5) 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.

Rice blast, caused by *Magnaporthe oryzae*, is one of the most disastrous diseases in rice-cultivating regions worldwide. It is also considered as a model system to understand interactions between fungal pathogens and host plants. During interactions between rice and *M. oryzae*, transcriptional profiles of pathogenicity and defense genes would be reprogrammed. Furthermore this hemibiorophic fungus has a lifestyle transition from biotrophy to necrotrophy during interactions. To decipher this intricately weaved interaction networks, we performed genome-wide transcriptional profiling of genes using dual RNA-seq approach. We collected infected rice cells from inoculated sheaths under microscope to enrich fungal samples during infection as a sequential manner. These include pre-penetration (16-18 hpi), biotrophic (34-26 hpi), and nectrotrophic (72 hpi) stages. Total of 12,559 and 39,983 transcripts were identified from *M. oryzae* and rice, respectively. Distance matrix and principle component analyses of total gene expression showed that three infection stages were closer than other stage. At each stage, 948, 752, and 949 stage specific transcripts were identified in *M. oryzae*. One hundred seventeen effector candidates were identified from functional annotation of these transcripts, and highly enriched in 36-38 hpi, whereas the transcripts for cell wall degrading enzymes were enriched in 72 hpi. In rice, 1,232, 592 and 3,542 stage specific transcripts were also identified from each stages. These data would provide a blueprint for genome-wide transcriptional reprogramming during rice-*M. oryzae* interactions.

289. Effects of argentilactone on the transcriptional profile, cell wall and oxidative stress of *Paracoccidioides* spp. F. Araújo[1], L. Coelho[1], B. Neto[1], J. Parente-Rocha[1], A. Bailão[1], C. Oliveira[2], G. Fernandes[3], O. Ruiz[4], J. Ochoa[4], C. Soares[1], M. Pereira[1]. 1) Laboratório de Biologia Molecular, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil; 2) Laboratório de Produtos Naturais, Instituto de Química, Universidade Federal de Goiás, Goiânia, Goiás, Brazil; 3) Laboratório de Biodados, Biologia Celular e Desenvolvimento, Universidade Católica de Brasília, 70790-160, Goiânia, Goiás, Brasil; 4) Unidad de Biología Celular y Molecular, Corporación para Investigaciones Biológicas (CIB) and Facultad de Medicina Universidad de Antioquia, Medellín, Colombia.

Paracoccidioides spp, dimorphic pathogenic fungi, is the etiologic agent of paracoccidioidomycosis (PCM). PCM is an endemic disease that affects at least 10 million people in Latin America, causing severe public health problem. The drugs used against pathogenic fungi have various side effects and have limited efficacy, therefore there is an inevitable and urgent medical needing for the development of new antifungal drugs. In the present study, we evaluated the transcriptional profile of *Paracoccidioides* spp exposed to argentilactone, a constituent of the essential oil of *Hyptis ovalifolia*. A total of 1,058 genes were identified, of which 208 were up-regulated and 850 down-

regulated. The cell rescue, defense and virulence, with a total of 26 genes, was a functional category with a large number of genes induced, among them, heat shock protein 90 (*hsp 90*), cytochrome c peroxidase (*ccp*), hemoglobin ligant RBT5 (*rbt5*) and superoxide dismutase (*sod*). Quantitative real-time PCR revealed an increase in the expression level of all those genes. Enzymatic assay showed a significant increase in SOD activity. The reduced growth of *Pbhsp90*-aRNA, *Pbccp*-aRNA, *Pbsod*-aRNA, *Pbrbt5*-aRNA isolates in the presence of argentilactone indicates the importance of these genes in *Paracoccidioides* spp responding to argentilacone. *Paracoccidioides* spp cell wall was also evaluated. The results showed that argentilactone causes a decrease in the levels of polymers of the cell wall. These results suggest that argentilactone is a potential candidate for antifungal therapy.

290. Comparative and transcriptional analysis of the predicted secretome in the lignocellulose degrading basidiomycete *Pleurotus ostreatus*. M. Alfaro[1], J.L. Lavín[1,2], R. Castanera[1], J.A. Oguiza[1], L- Ramírez[1], A.G. Pisabarro[1]. 1) Genetics and Microbiology Research Group, Pub Univ Navarra, Pamplona, Navarre, Spain; 2) Genome Analysis Platform, CIC bioGUNE & CIBERehd, 48160 Derio, Spain.

Fungi interact with their environment by means of secreted proteins that obtain nutrients, elicit responses and modify their surroundings. As the set of proteins secreted by a fungus is influenced by its lifestyle, it should be possible to use it as a tool to predict the lifestyle of a given basidiomycete fungus. To test this hypothesis, we have identified bioinformatically the set of secretable proteins in two monokaryotic strains (haplotypes) of the white rot basidiomycete *Pleurotus ostreatus* (PC9 and PC15) using the web based pipeline SECRETOOL. We identified 538 and 554 protein models to be secreted (4.41% and 4.77% of the PC9 and PC15 gene models, respectively). The functional annotation of the proteins predicted to be secreted revealed the unknown (37.2%), glycosyl hydrolases (26.5%) and red-ox enzymes (11.54%) as the main functional groups. Furthermore, the distribution of these groups was nearly identical in the two strains. Then, we combined the predicted secretome data with two RNA-seq analyses to study the relationship between the functional profiles of the predicted secretome and the expression level of each group. We found that the relative importance of the groups was deeply modified (being the relevance of the unknown group further enhanced) and that the transcriptome profile of secreted proteins was significantly different in the two analyzed strains (revealing their different functional responses to the same environment). Finally we used the set of the proteins secreted by this white rot model as a query to search the secreted proteins in the fungal genomes released in the JGI Mycocosm, and we found that their secretome profiles could be used to cluster them into groups coherent with their particular lifestyles rather than with their corresponding phylogenetic positions.

291. Interspecific interactions between saprotrophic Agaricomycetes, the associated gene expression and the impact on the wood decay community. G. Powell[1], L. Boddy[2], E. Dudley[1], J. Hiscox[2], M. Savoury[2], S. Moody[1], D. Eastwood[1]. 1) Swansea University, Department of Bioscience, Wallace Building, Swansea, United Kingdom; 2) Cardiff University, Cardiff School of Biosciences, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX.

Saprotrophic basidiomycetes are the major decomposition agents in temporal and forest ecosystems, facilitating carbon and nutrient cycling, as well as healthy ecosystem functioning. Interactions occur as fungi compete for limited resources, and are crucial determinants of community development within decaying wood, affecting succession of species and substratum decay rate. Certain species are able to form extensive networks of aggregated hyphae (cords), which can extend from a colonised resource to forage, establishing antagonistic interactions when they encounter new resources colonised by other mycelia. Mechanisms utilised during combat include chemical signalling, extracellular enzyme and secondary metabolite activity, and changes in mycelial morphology. Previous microarray studies on agar have identified relationships between changes in gene expression and interaction outcome, highlighting the importance of further investigation of the transcriptome during interactions on ecologically relevant substrates. Beech (*Fagus sylvatica*) wood blocks were pre-colonised with single wood decay fungi from different stages of the successional community (hence, with different combative abilities). Pairwise interactions were set up by placing two blocks, each colonised with a species that uses different foraging strategies (one cord forming and one non-cord forming), separately onto the surface of soil microcosms; interaction zones were established when mycelial cord growth from the cord forming competitor contacted the non-cord former block. Wood blocks and mycelia were harvested at four stages during the interaction, prior to RNA and protein extractions. In this project molecular responses associated with fungal interactions on soil will be identified using RNA-seq to profile transcriptional changes over time, and supported by proteomics. Subsequently, functional studies of key genes identified will expedite understanding of these complex interactions and processes.

292. Comparative analysis of transcriptomes and secretomes of the white-rot fungus *Dichomitus squalens* cultured in lignocellulosic substrates. Johanna Rytioja[1], Miaomiao Zhou[2,3], Kristiina Hildén[1], Marcos Di Falco[4], Outi-Maaria Sietiö[1], Adrian Tsang[4], Ronald P. de Vries[2,3], Miia R. Mäkelä[1]. 1) Food and Environmental Sciences, University of Helsinki, Helsinki, Finland; 2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; 3) Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands; 4) Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada.

White-rot fungi are essential organisms in the degradation of plant biomass and thus play an important role in the global carbon cycle. In addition to their ability to mineralize the aromatic lignin polymer, white-rot fungi produce enzymes that degrade plant cell wall polysaccharides into oligosaccharides and monomeric sugars that they can use as carbon source. These enzymes are important tools for biotechnology, as the products of their catalysis can be used as precursors of bio-based fuels and chemicals.

Dichomitus squalens is a white-rot basidiomycete capable of efficient cellulose and lignin degradation on softwood. The focus of this work was to dissect the enzyme combinations used by the *D. squalens* in the degradation of different types of plant-derived biomass. For that, *D. squalens* was grown on hardwood, softwood, and residues from monocots and dicots as carbon sources and sampled at two time points for transcriptome and secretome analyses. Extracellular plant biomass degrading enzyme activity profiles were also determined during the growth of *D. squalens* on these plant biomass materials. These analyses provide an in-depth appreciation of the mechanisms used by *D. squalens* in the degradation of diverse plant-derived biomass. Further, these results are also expected to enhance the discovery and use of novel enzymes in biotechnological applications.

293. High-efficiency genome editing and allele replacement in prototrophic and wild strains of *Saccharomyces*. William Alexander[1,2], Drew Doering[1,3], Chris Hittinger[1,2,3]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI; 3) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

Current genome editing techniques available for *Saccharomyces* yeast species rely on auxotrophic markers, limiting their use in wild and industrial strains and species. Taking advantage of the ancient loss of thymidine kinase in the fungal kingdom, we have developed the herpes simplex virus thymidine kinase gene as a selectable and counterselectable marker that forms the core of novel genome engineering tools called the Haploid Engineering and Replacement Protocol (HERP) cassettes. Here we show that these cassettes allow a researcher to rapidly generate heterogeneous populations of cells with thousands of independent chromosomal allele replacements using mixed PCR products. We further show that the high efficiency of this approach enables the simultaneous replacement of both alleles in diploid cells. Using these new techniques, many of the most powerful yeast genetic manipulation strategies are now available in wild, industrial, and other prototrophic strains from across the diverse *Saccharomyces* genus.

294. Signature gene expressions of cell wall integrity pathway concur with tolerance response of industrial yeast *Saccharomyces cerevisiae* against biomass pretreatment inhibitors. Z. Lewis Liu. USDA-ARS National Center for Agricultural Utilization Research, Peoria, IL.

Traditioanl industrial ethanologenic yeast *Saccharomyces cerevisiae* has a robust performance under various environmental conditions and can be served as a candidate for the next-generation biocatalyst development for advanced biofuels production using lignocellulose mateials. Overcoming toxic compounds liberated from lignocellulosic biomass pretreatment is among numerous challenges for a sustainable biofuels industry. In a genomic expression study for an industrial type strain NRRL Y-12632, key genes involved in cell wall integration signaling pathway, including *WSC2, WSC3, FKS1, FSK2, PLC1, PKC1, BCK1, MKK1, MLP1, MLP2, RLM1, SWI4,* and *SWI6*, were found to display distinct enhanced signature expressions in response to 30 mM 5-hydroxymethyl-2-furaldehyde (HMF), a commonly encountered biomass pretreatment inhibitory compound. Within 2h after HMF treatment, expressions of these genes increased to 3- to 4-fold higher comparing with an untreated control. Examination of most genes using analogous single-gene deletion mutations showed that all of these knock out mutants grew normally on a synthetic medium but failed to grow on the medium containing 20 mM HMF. These results suggest an essential role for each of these genes against HMF challenges. Since this tolerance response is adaptive, the cell wall integrity signaling pathway is likely involved in mediation to the tolerance of the industrial yeast against pretreatment inhibitors, in addition to other major regulatory elements. Sequence comparison of these genes from the industrial strain Y-12632 also revealed at least 42 non-synonymous SNPs, resulting in amino acid sequence substitutions, comparing with the reference model strain S288C using PCR sequencing analysis. The industrial yeast strain constantly shows more tolerance and adaptive responses to a wide range of inhibitory compounds than the model strain S288C. Variations in genomic sequence for the industrial yeast including concentrated SNPs involved in the cell wall integrity signaling pathway could attribute to this tolerance response.

295. Hosting multiple fungal genomes to investigate the phylogenetic distribution of virulence factors. Ulrich Güldener[1,2], Christian Sieber[2], Mathias Walter[1], Martin Münsterkötter[2]. 1) Department of Genome oriented Bioinformatics, Technische Universität München, Freising, Germany; 2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany.

The Fungal and Microbial Genomics group at IBIS, Helmholtz Zentrum München, is rooted in the annotation project of yeast, the first sequenced eukaryotic genome. Since then, the group started to annotate and analyse the filamentous model eukaryote *Neurospora crassa* and subsequently worked on the genomes of rusts, plant pathogenic Ascomycetes, Basidiomycetes and yeasts. The current work includes the assembly of genomes based on NGS data and employment of RNA-seq data for structural annotation and expression analysis. Combining these evidences with functional annotation resulted in the detection of known and yet unknown potential secondary metabolite (SM) clusters in Fusarium clades and other species which allows to investigate the phylogenetic distribution of the predicted clusters in the fungal kingdom. For example, the recent analysis on the *F. graminearum* SM potential revealed 67 secondary metabolite clusters and hints of horizontal gene cluster transfer. To enable cross-kingdom comparisons, the genomic data and annotation of currently 300 fungal genomes are maintained in genome databases which are connected to genome- and synteny browsers for specific subsets of genomes. All protein sequences are submitted to the Similarity Matrix of Proteins (SIMAP) which is used as similarity and ortholog information resource. With these resources at hand a comprehensive analysis of SM clusters and further virulence factors like secreted effectors is feasible. The databases of the 300 fungal genomes provide a comprehensive set of bioinformatics methods results and automatic functional classification using FunCat and GO. The users can retrieve the functional distribution of their lists of genes for all genomes maintained (e.g. a set of co-regulated genes) using the functional classification of the annotated genes and proteins. http://www.helmholtz-muenchen.de/en/ibis/institute/groups/fungal-microbial-genomics/resources/index.html.

296. Examining the evolution of the regulatory circuit controlling secondary metabolism and development in *Aspergillus*. Abigail Lind[1], Jennifer Wisecaver[2], Timothy Smith[3], Xuehan Feng[3], Ana Calvo[3], Antonis Rokas[1,2]. 1) Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Biological Sciences, Vanderbilt University, Nashville, TN; 3) Biological Sciences, Northern Illinois University, Dekalb, IL.

Filamentous fungi produce diverse secondary metabolites (SMs) essential to their ecology and adaptation. Although each SM is typically produced by only a handful of species, global SM production is governed by widely conserved transcriptional regulators in conjunction with other cellular processes, such as development. We examined the interplay between the taxonomic narrowness of SM distribution and the broad conservation of global regulation of SM and development in *Aspergillus*, a diverse fungal genus whose members produce well-known SMs. Evolutionary analysis of the 2,124 genes comprising the 262 SM pathways in four *Aspergillus* species showed that most SM

pathways were species-specific, that the number of SM gene orthologs was significantly lower than that of orthologs in primary metabolism, and that the few conserved SM orthologs typically belonged to non-homologous SM pathways. RNA sequencing of two master transcriptional regulators of SM and development, *veA* and *mtfA*, showed that the effects of deletion of each gene, especially *veA*, on SM pathway regulation were similar in *A. fumigatus* and *A. nidulans*, even though the underlying genes and pathways regulated in each species differed. In contrast, examination of the role of these two regulators in development, where 94% of the underlying genes are conserved in both species showed that whereas the role of *veA* is conserved, *mtfA* regulates development in the homothallic *A. nidulans* but not in the heterothallic *A. fumigatus*. Thus, the regulation of these highly conserved developmental genes is divergent, whereas–despite minimal conservation of target genes and pathways–the global regulation of SM production is largely conserved. We suggest that the evolution of the transcriptional regulation of secondary metabolism in *Aspergillus* represents a novel type of regulatory circuit rewiring and hypothesize that it has been largely driven by the dramatic turnover of the target genes involved in the process.

297. A software tool for visual analysis of secondary metabolism gene clusters. Akira Ohyama[1,4], Myco Umemura[2,4], Itaru Takeda[3], Takashi Kubo[4], Makoto Matsui[4], Yuki Miyamura[4], Kaoru Nemoto[4], Tomoko Ishii[4], Masayuki Machida[2,4]. 1) in silico biology, inc., Yokohama, Japan; 2) National Institute of Advanced Industrial Science and Technology (AIST), Sapporo/Tsukuba, Japan; 3) Tokyo University of Agriculture and Technology, Tokyo, Japan; 4) Technology Research Association of Highly Efficient Gene Design, Tokyo, Japan.

We developed software tools for the motif-independent prediction of secondary metabolism biosynthesis (SMB) gene clusters, MIDDAS-M[1] and MIPS-CG[2]. Recently, MIDDAS-M successfully detected the gene cluster for the biosynthesis of ustiloxin B, a circular peptide compound which possesses N-methylation and a norvaline moiety. This was the first finding of RiPS (Ribosomal Peptide Synthesis) pathway from filamentous fungi[3]. Once SMB gene cluster are predicted, their accuracy and function are to be experimentally evaluated by mobilizing knowledge of SMB genes, especially those other than so-called "Core Genes" such as PKS and NRPS especially for those of novel SMB pathways such as RiPS. We have developed in silico MolecularClonig Genome Design Suite (IMCDS) to effectively design the validation experiments. IMCDS allows visual analysis and comparison of predicted and known SMB gene clusters under multiple representation capability including gene expression from genome-wide to nucleotide sequence views. It includes automatic color visualization of genes based on their functional categories and designing primers for the preparation of a DNA fragment to disrupt a target gene.

1. Umemura et al., PLoS One 8, e84028 (2013).
2. Takeda et al., DNA Res (2014).
3. Umemura et al., Fungal Genet Biol (2014).

298. Novel Comparative Genomics Approach for Motif-Independent Prediction and Similarity Analysis of a Secondary Metabolism Gene Cluster. Itaru Takeda[1,2], Myco Umemura[2], Hideaki Koike[2], Kiyoshi Asai[3,4], Masayuki Machida[1,2]. 1) Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan; 2) Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba/Sapporo, Japan; 3) Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo, Chiba, Japan; 4) Computational Biology Research Center (CBRC), AIST, Tokyo, Japan.

Despite biological importance, many genes for secondary metabolite biosynthesis (SMB) remain unspecified. It is due largely to their high sequence diversity and silence in expression under a general cultivation conditions. Some software tools including SMURF and antiSMASH have been developed to predict fungal SMB gene clusters by detecting core genes encoding non-ribosomal peptide synthetase, polyketide synthase and so on as well as several typical accessory genes, those encoding transcription factors and transporters, for example,. In this study, we have devised a novel prediction method of SMB gene clusters (MIPS-CG) by a comparative genomics approach, allowing motif-independent prediction. The gene cluster pairs were first detected based on similarity of the order of genes with high sequence similarity, followed by adjustment of cluster margins and exclusion of clusters in syntenic regions. By varying several parameters in the procedure above, we have successfully realized high detection sensitivity as well as accurate prediction of cluster margins. Our method allowed detection of 21 out of 24 known SMB gene clusters in the genome sequences of 10 filamentous fungi including the kojic acid biosynthesis gene cluster of *Aspergillus oryzae*. The result indicated that a pair of gene clusters was detected largely by similar gene contents rather than order of the genes. This means that our method is applicable to the detection of gene cluster pairs even having significant rearrangement possibly occurred during evolution from a common ancestral gene cluster. Furthermore, a significant difference in the sequence characteristics was found between the genes residing inside the clusters and those outside the clusters by a comparison between sets of the genes inside and outside the clusters.

299. Clues to an evolutionary mystery: the genes for T-toxin, enabler of the devastating 1970 Southern Corn Leaf Blight epidemic, are present in ancestral species. Bradford Condon[1], Candace Elliott[2], Sung-Hwan Yun[3], Youhei Haruki[4], Motochiro Kodama[4], B. Gillian Turgeon[1]. 1) Department of Plant Pathology, Cornell University, Ithaca, NY; 2) School of Botany, The University of Melbourne, Parkville 3010 VIC, Australia; 3) Department of Medical Biotechnology, Soonchunhyang University, Asan, Chungnam 336-745, South Korea; 4) Laboratory of Plant Pathology, Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan.

The Southern Corn Leaf Blight epidemic of 1970 devastated fields of T-cytoplasm corn planted in monoculture throughout the eastern US. The epidemic was driven by race T, a previously unseen race of the fungal Dothideomycete pathogen, *Cochliobolus heterostrophus.* Race T produces T-toxin, a polyketide host selective toxin encoded by the genetically complex *Tox1* locus. Despite forty years of research, a definitive inventory of the genes at *Tox1* and their evolutionary origin have remained a mystery. Here we show that an Eurotiomycete species, *Penicillium raistrickii*, and two additional Dothideomycete species, *Corynespora cassiicola* and *Leptosphaeria*

maculans, possess all of the known *Tox1* genes, at a single collinear genetic locus. The compact gene clusters in these species are in stark contrast to the genetically disjointed *C. heterostrophus* race T *Tox1* locus, and this finding suggests that the race T arrangement cannot be the ancestral state. Furthermore, the clusters facilitate the definition of the *Tox1* genetic boundaries. The *Tox1*-like gene discovery in other species, especially in an Eurotiomycete, offers an opportunity to tackle the *Tox1* evolutionary timeline for the first time, as the Eurotiomycete/Dothideomycete split is estimated to have occurred ~320-400 MYA. However, phylogenetic analyses of PKS-encoding genes are difficult because such genes have rapid evolution signatures and notoriously discontinuous distribution patterns. Thus, despite finding *Tox1*-like gene clusters in species ancestral to *C. heterostrophus*, we find it difficult to distinguish between a history of discontinuous evolution mediated by loss, gain, and recombination and a history of horizontal gene transfer.

300. Using next generation sequences to study plant-pathogen emergenceUsing next generation sequences to study plant-pathogen emergence. Elisha Thynne, Megan McDonald, Peter Solomon. Plant Sciences, Australian National University, Canberra, ACT, Australia.

In recent years, both individual genes and whole genome sequences for different fungal species have become ubiquitous on sequence-databases. Increased access to gene sequences has heightened our ability to quickly and effectively study emerging fungal pathogens. The gene sequences of a recently emerged pathogen can be compared to a wide range of species, allowing for unprecedented speed of accurate identification. Similarly, comparison to whole genomes from a range of seemingly disparate organisms can provide insight into how a pathogen has emerged.

We have begun to utilize these resources to better understand a recently emerged phytopathogen complex that cause the disease known as white grain disorder (WGD) in wheat. Original reports on the disease described the causal agent as *Botryosphaeria zeae*. As part of a broader study into WGD in this laboratory, we have sequenced the genomes of WGD pathogens and have reclassified these fungi to a different *Botryosphaeriaceae* genus, *Tiarosporella*. Furthermore, with the aid of a multigene-phylogeny, we have delimited wheat-infecting *Tiarosporella spp*. into three separate species.

Currently it is unknown how wheat-infecting *Tiarosporella spp.* are able to infect and cause disease. Using the sequenced the genomes of the three species, we are endeavoring to identify genes of interest that may potentially play a role in virulence. One such gene is predicted to encode a large, 56kb modular polyketide synthase (PKS). Modular PKS genes have traditionally only been found in bacteria and we have only identified one other homologue in a different fungus. Interestingly, this other fungus is also a member of the *Botryosphaeriaceae* family, and a major agricultural pathogen. We believe this gene may have been obtained via horizontal gene transfer at some point during these fungi's evolution, perhaps from a bacterial origin. We aim to characterise the SM product of this predicted protein, and determine whether it is a virulence factor for these fungi.

301. Rearrangements of the *MAT1* gene cluster architecture in the genus *Calonectria* . Martha Malapi-Wight[1], Daniel Veltri[1,2], Yazmin Rivera[1,2], Jo Anne Crouch[1]. 1) Systematic Mycology & Microbiology Lab, USDA-ARS, Beltsville, MD; 2) Department of Plant Biology & Pathology, Rutgers University, New Brunswick, NJ.

Calonectria pseudonaviculata and *C. henricotiae* (Ascomycetes: Sordariomycetes) are the causal agents of the boxwood blight disease. In the past fifteen years, these destructive fungal pathogens have spread worldwide, including the 2011 entry of *C. pseudonaviculata* into the U.S. In this work we sequenced nineteen *Calonectria* genomes, including fifteen isolates of *C. pseudonaviculata and C. henricotiae*, and representatives of *C. indonesiae, C. naviculata* and *C. leucothoes*. Genome sizes ranged from 53−69.8 Mb, with coverage depths from 42X−297X, N50 ranges from 13−253 Kb, and GC contents between 46−51%. Draft genome assemblies were used to identify and assess the determinant of mating, the *MAT1* gene. Only a single *MAT1* idiomorph was identified in each species, indicating that they utilize heterothallic mating systems. *Calonectria henricotiae* and *C. naviculata* possessed a *MAT1*-1 idiomorph, while *C. pseudonaviculata, C. indonesiae* and *C. leucothoes* showed a *MAT1*-2 genotype. Overall, the length of the *MAT1-1* loci was 4.2 Kb, and the length of *MAT1-2* ranged from 3.1−3.3 Kb. In contrast to what has been observed within genera in the Ascomycota, syntenic mapping revealed dramatic rearrangements of the *MAT1* gene cluster architecture between the five *Calonectria* species. Only a block of four genes located immediately adjacent to the *MAT1* gene were syntenic between species. Genes located further upstream and downstream of the *MAT1* idiomorph have gone through major gene rearrangements. Population analysis of the two boxwood-associated species revealed that *C. pseudonaviculata*/*MAT1-2* genotype is present worldwide, whereas *C. henricotiae/MAT1-1* genotype is only present in five European countries. This work provides the first comprehensive analysis of the genetic structure, distribution and syntenic analysis of the mating-type loci of the *Calonectria* genus. It also provides an understanding of the boxwood blight fungi sexual reproductive potential, which holds strong implications for risk assessment, disease epidemiology, and the respective development of effective control strategies.

302. Investigation of protein phosphatases in *Trichoderma reesei*. Aroa Rodriguez Iglesias, Monika Schmoll. Austrian Institute of Technology GmbH, Health & Environment, Bioresources, Konrad-Lorenz-strasse 24, 3430 Tulln, Austria.

Perception of external environment changes and detection of intracellular energetic status allows the balance of requirements for growth and cell survival. Cellular responses are regulated by different processes as post-translational modifications by phosphorylation and dephosphorylation. Protein kinases (PKs) and phosphatases (PPs) play a key role in signal transduction pathways in eukaryotic cells via modulating protein phosphorylation state and activity, hence coordinating subsequent responses. Nevertheless, although protein kinases have been extensively studied, little is known about protein phosphatases as key components of the cellular signaling machinery to maintain a proper balance in phosphorylation cycles. Protein phosphatases (PPs) are traditionally classified on the basis of substrate specificity as serine/threonine (PSP), tyrosine (PTP), dual-specificity (DSP), or histidine PPs. In the genome of *T. reesei* 38 genes encoding protein phosphatases have been annotated, with 20 belonging to PSP, 12 to PTP and 3 to DSP. Additionally, 2 phosphotyrosyl phosphatase activators (PTPA) and one phosphatase inhibitor were detected. 16 knock-out strains of non-essential protein phosphatases in *T. reesei*

were generated in order to characterize the role of those proteins. We found involved in regulation of hyphal extension rate, cellulase activity, protease production, growth rate, growth on different carbon sources and growth under osmotic or oxidative stress in light and/or darkness. Also differences in sexual development and light responsive conidiation were observed. A hierarchical cluster analysis of transcript patterns of phosphatase genes in different mutants upon growth on cellulose or growth of the wildtype on inducing or repressing carbon sources was compared to phenotypic analysis performed for the knock-out strains. The results showed that groups of protein phosphatases clustered according to a correlation between the transcript patterns and phenotype. Consequently, the first insights provided by the transcriptome data for some groups of protein phosphatases in *T. reesei* were confirmed in consonance with observed phenotypes.

303. A high throughput system to uncover the function of predicted genes in *Trichoderma virens*. Guillermo Nogueira Lopez, Sarah Lyne, Maria Fernanda Nieto Jacobo, Artemio Mendoza Mendoza. Bio-Protection Research Centre, Lincoln University, Christchurch, Lincoln, New Zealand.

In nature, almost every single plant is colonised by fungi that cause no disease symptoms. These beneficial fungi which colonise internal tissues, called endophytes, are widely studied because of their properties as biocontrol and biofertilizer agents. Among these microorganisms the genera *Trichoderma* is an important model to study plant-endophyte interactions. A large number of *Trichoderma* species behave as opportunistic-endophytic plant symbionts and have the capacity to penetrate plant tissues in order to create a beneficial interaction. Despite the fact that most plants are colonized by fungal endophytes, there are few studies focusing on understanding the molecular mechanisms which determine these mutual interactions. Next-generation sequencing technology coupled with high throughput tools for functional genomics (e.g. protein localization, gene deletion and protein targeting) is desired for a better understanding of *Trichoderma*'s biology. Here we present the creation of specific vectors for fungal gene deletions (hygromycin phosphotranferase), protein localization (mCherry, 3X-eGFP, eGFP), overexpression (constitutive and inducible promoters) and protein targeting (HA, C-myc and FLAG tags) by using the Golden gate shuffling technology. Additionally, we generated a novel selection marker base in carboxin resistance and a non-homologous end-joining-deficient strain (delta- ku70) in *Trichoderma virens* Gv29.8. In this presentation we will discuss the advantages and disadvantages of our high throughput system.

304. Genomic analyses of *Mortierella elongata* and associated bacterial endosymbiont (Candidatus *Glomeribacter* sp.). Jessie Uehling[1], Gregory Bonito[1], Khalid Hameed[1], Jessy Labbe[2], Dale Pelletier[2], Timothy Tschaplinski[2], Amy Schaefer[3], Christopher Schadt[2], Francis Martin[4], Rytas Vilgalys[1]. 1) Duke University, Durham, NC; 2) Oak Ridge National Laboratory, Oak Ridge, TN; 3) University of Washington, Seattle, WA; 4) Institut National de la Recherche Agronomique, Nancy, Lorraine, France.

Recently efforts to understand *Populus* microbiome dynamics have yielded cultures of many plant-beneficial fungi, including several *Mortierella elongata* (*Mortierellomycotina*) isolates. *M. elongata* associates with diverse rhizosphere bacteria that alter growth and function of their fungal associates. Some bacteria are free-living around root tips and hyphae while others are confined to living inside of fungal hyphal cells. The ecological strategies of these bacteria living inside of fungal cells fall along a spectrum from facultative to obligate endosymbiosis. While bacterial endosymbiont presence and identity can be established with next generation sequencing, especially in obligate symbionts these systems are challenging as conditions for growth of the bacteria are unknown. Experimental tractability can be gained by clearing bacteria from fungi with antibiotics, and comparating growth and metabolite accumulation of cleared and uncleared strains. Our research goal is to use multiple -omics approaches to analyze interaction dynamics between *M. elongata* and its endosymbiotic bactiera to answer the following questions; ***1.*** *What effect on fungal health and functioning do these bacteria impart?* ***2.*** *What factors dictate assembly and function of hyphal endosymbiont communities in the* Populus *rhizosphere?* We used 454 pyrosequencing, antibiotic passaging, radial growth assays, and gas chromatography coupled with tandem mass spectrometry to address these questions. Identifying functional attributes of the fungal microbiome and rhizosphere microbial interactions will ultimately enable study of communities with maximal synergistic effects on plant growth, stress tolerance, and fitness.

305. Responses to hypoxia in *Paracoccidioides* sp. P. Lima[1], D. Chung[2], R. Cramer[2], C. Soares[1]. 1) Molecular Biology, Universidade Federal de Goias, Goiania, Goias, Brazil; 2) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA.

The genus *Paracoccidioides* represents the causative agents of paracoccidioidomycosis, a systemic mycosis in Latin America. To survive in the human body, pathogens must adapt to microenvironments, which are often characterized by hypoxia (significantly low levels of oxygen). The oxygen is a critical factor to eukaryotic cells determining overall cellular metabolism. The responses of the *Paracoccidioides* sp., *Pb*01 strain, to hypoxia is relevant in this context. A comprehensive response of *Pb*01 submitted to hypoxia was accessed by transcriptional (qRT-PCRs), proteomic (NanoUPLC-MSE) and genetic complementation approaches. The results revealed that adaptability of *Pb*01 to hypoxia is possibly mediated by a highly conserved transcription factor, SrbA, a protein of the sterol regulatory element binding protein family. The *PbsrbA* is functional in a null mutant of *srbA* of *A. fumigatus* (*ΔsrbA*) and restore both, the susceptibility of *ΔsrbA* to the azoles and the biomass production in response to low iron conditions. In addition, transcriptional analysis of the *Pb*01 yeast cells under hypoxia, showed that *Pb*01 increases the expression of transcripts associated with glycolysis and ergosterol/ fatty acid biosynthesis. The fungus also regulates the abundance of proteins when was submitted to hypoxia for 12 and 24 h. A total of 310 proteins were regulated at both time points. Proteins involved in acetate and lactate were up regulated under hypoxia. Ergosterol precursor molecules such as acetyl-CoA and acetoacetyl-CoA were also increased. In addition, proteins from glycolysis, GABA shunt and those involved in vesicular/ vacuolar transport were up represented. On the other hand, representatives of the tricarboxylic acid pathway, acetyl-CoA biosynthesis, pentose phosphate pathway and cations/ metals transport had decreased abundance in our analysis. The characterization of the responses of *Pb*01 to oxygen deprivation is important to elucidate molecules and processes to understanding the fungal establishment in the host.

306. Comparative analysis of transcription factors families across fungal tree of life. Asaf Salamov, Igor Grigoriev. DOE Joint Genome Institute, Walnut Creek, CA.

Transcription factors (TFs) are proteins that regulate the transcription of genes, by binding to specific DNA sequences. We analysed the distribution and evolution of 60 known TF families in more than 300 fungal genomes from Mycocosm portal (http://jgi.doe.gov/fungi/). We have shown that while TF families, unique to fungal kingdom , like Zinc finger Zn2Cys6 and fungal-specific TFs, make up the largest fraction of TFs repertoire in most fungal genomes, especially greatly expanding in Pezizomycotina clade of Ascomycota, the universal eukaryotic TFs, like HLH, Homeobox, bZIP, GATA and others, are more abundant in Zygomycota and other early-divergent clades. We discuss the different evolutionary pathways of individual TF families.

307. Comparative transcriptome analysis of *Aspergillus fumigatus* conidia reveals a novel secondary metabolism gene cluster specifically expressed at low temperature. D. Hagiwara[1], K. Sakai[1], S. Suzuki[2], K. Kamei[1], T. Gonoi[1], S. Kawamoto[1]. 1) Medical Mycology Research Center, Chiba university, Chiba, Japan; 2) National Food Research Institute, Ibaraki, Japan.

Filamentous fungi vigorously produce asexual spores (conidia) under appropriate conditions. Conidia are reproductive structures that are important for both distribution and survival for fungi. To understand effects of culture temperature on tolerance of conidia to various stresses, we compared sensitivities to heat, hydrogen peroxide, and UV irradiation among *Aspergillus fumigatus* conidia harvested from cultures at different temperatures (25, 37, and 45°C). The conidia from 25°C-culture showed a lower tolerance to heat stress (60°C) and to oxidative stress (H_2O_2) compared with the other conidia, and showed a marked resistance to UV stress. We found that accumulation of trehalose, which plays a protective role in heat stress, was reduced in the conidia from 25°C-culture. Furthermore, the color of conidia from 25°C-culture was darker than those from 37 and 45°C-cultures, suggesting an increased melanin on the surface of conidia from 25°C-culture.

To gain more insight into temperature-specific accumulation of secondary metabolites other than melanin in conidia, we investigated the transcriptome in the conidia from 25°C-, 37°C-, and 45°C-cultures. The transcriptome data revealed that melanin biosynthesis gene cluster was increasingly expressed in the conidia from 25°C-culture, supporting the above hypothesis. We also found one novel secondary metabolite gene cluster, which showed a higher expression in the conidia from 25°C-culture compared to those from 37°C- and 45°C-culture. This cluster contains 13 genes including genes encoding a PKS and a C6-type transcription factor (AfAflR) homologous to aflatoxin biosynthesis regulating AflR. In the AfaflR gene deletion mutant, expression levels of 11 genes of the cluster were greatly reduced, suggesting that the AfAflR plays a central role in transcriptional regulation for this cluster. We are going to identify the metabolite produced from this cluster at low-temperature, and to discuss temperature-dependent secondary metabolite production in the conidia of the pathogenic fungus.

308. Bacteria induce defense in the fungus *Coprinopsis cinerea*. Anja Kombrink, Martina Stöckli, Markus Aebi, Markus Künzler. Microbiology, ETH Zürich, Zürich, Switzerland.

Fungi occur in diverse ecological habitats, growing as saprophytes on dead organic matter or establishing symbiotic interactions with plants or animals. In any niche, fungi encounter microbes that compete for nutrients and/or have antagonistic activity. While defense of plants and animals is well studied, little is known about defense mechanisms that fungi employ to cope with competitors and parasites. We investigate the defense mechanisms of the basidiomycete *Coprinopsis cinerea* towards various antagonistic organisms, including bacteria. Previous experiments have demonstrated that genes encoding nematotoxic proteins are upregulated in *C. cinerea* vegetative mycelium challenged with fungivorous nematodes. However, the response of *C. cinerea* to bacteria, which might involve induction and secretion of putative anti-bacterial effector proteins, needs further investigation. To this end, we compared the transcriptome of *E. coli*-challenged *C. cinerea* with unchallenged mycelium. The mycelium was grown on glass beads submerged in liquid medium to allow close and dynamic contact of the bacteria with the fungal hyphae. Eight hours after addition of the bacteria, a set of genes was found to be highly induced (>eight fold compared to the unchallenged control) in *C. cinerea* mycelium indicative of a response to the bacteria. Among the highly induced genes are eight members of a gene family that encodes eleven small cysteine-rich hypothetical proteins (SCRP) and four lysozymes whose N-termini share high homology with the SCRPs. The induction of these genes has been confirmed by quantitative RT-PCR and representatives of both lysozyme- and SCRP-encoding genes are cloned for protein production in order to further investigate their activity in defense against bacteria. Furthermore, we found that also filter-sterilized growth medium of an *E. coli* culture induces expression of the putative defense genes in *C. cinerea*. We are aiming at the identification of the bacterial cue that is perceived by the fungus and leads to this response.

309. Reliable transformation system for *Microbotryum lychnidis-dioicae* informed by genome and transcriptome project. Su San Toh[1], David Treves[2], Michael Perlin[1]. 1) Dept Biol, Program on Disease Evolution, Univ Louisville, Louisville, KY; 2) Indiana University Southeast, New Albany, Indiana, USA.

Microbotryum violaceum is a fungal species complex of related smuts primarily infecting members of the Caryophyllaceae (pinks). These smut species are limited to successful reproduction only on specific host species. The species complex has historically been fertile ground for investigations ranging from classical genetic studies to ecology of host/pathogen interactions. More recently, *M. violaceum* has become a model for emerging infectious disease through host shifts. Nevertheless, molecular genetic approaches towards understanding these aspects of the fungus have lagged behind. For instance, DNA sequence information has been collected only piecemeal over the last 10-15 years; also, reliable and reproducible means of transformation for these species has been lacking. To address these deficiencies, DNA from a haploid strain of *M. lychnidis-dioicae* on the host *Silene latifolia* was used to produce the draft genome sequence in collaboration with the Broad Institute. Using RNA-Seq, this collaboration also generated 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. Such RNASeq analysis allowed us to identify genes preferentially expressed at high levels under different conditions of growth. Combining the genome and transcriptome

data provided candidate regions upstream of genes to drive expression of target genes. As success in transforming recalcitrant basidiomycetes and Pucciniomycotina yeasts has employed *Agrobacterium*-mediated protocols, we adapted such a vector by fusing a hygromycin resistance cassette to a putative *M. lychnidis-dioicae* promoter from a gene highly expressed by haploid cells under rich nutrient conditions. Moreover, as proof-of-principle experiments to test predictions from our RNA-Seq data, we fused the eGFP gene to putative promoters for high-level expression under different growth conditions. The resulting transformants provide promising possibilities for both transformation and over-expression of genes in this fascinating biological system.

310. Comparative transcriptome analysis reveals different mechanisms of host adaptation of *Sporisorium reilianum* to maize and sorghum. Alana Poloni[1,2], Jan Schirawski[1,2]. 1) Microbial Genetics, RWTH Aachen University, Aachen, Germany; 2) Molecular Biology of Plant-Microbe Interaction, Albrecht-von-Haller Institute, Georg-August-University, Goettingen, Germany.

The biotrophic basidiomycete *Sporisorium reilianum* exists in two host-adapted formae speciales that cause head smut on sorghum (*S. reilianum f. sp. reilianum*; SRS) or on maize (*S. reilianum f. sp. zeae*; SRZ). To elucidate how host specificity of *S. reilianum* is established, we compared infection progression, plant defense reactions and host transcriptome responses of both fungi on maize and sorghum. On sorghum, SRS penetrated seedling leaves, spread into nodes and meristems, and induced sorus formation at the place of the inflorescence. SRZ was able to penetrate and multiply in sorghum leaves but did not reach nodes or meristems. Instead, strong plant defense reactions were induced that included H_2O_2 formation, callose deposition and phytoalexin induction. On maize, both fungi were able to penetrate, spread, and reach nodes and meristems, but only SRZ was able to form spores in the inflorescences. Plant defense reactions were very weak for both fungi on maize. Transcriptome analysis revealed a set of commonly regulated genes on either maize or sorghum, as well as many plant genes that were specifically induced by one forma specialis only. SRZ specifically induced a panoply of sorghum defense genes including genes involved in innate immune response, phenylpropanoid biosynthesis, and chitinase activity, whereas SRS specifically induced plant cell-replication genes including genes involved in mitotic spindle, ribosome, translation, DNA replication, lipid localization, chromatin remodeling, and sorghum cell wall modification enzymes. On maize, both fungi induced specific maize genes belonging to the same classes, like oxidoreduction and iron binding. In addition, SRS remarkably induced a suite of pentatricopeptide proteins of unknown function. Adaptation of *S. reilianum* to a compatible host seems to require different mechanisms in maize and sorghum.

311. Genome annotation and transcriptome analysis of smut fungi reveals widespread intergenic transcription and conserved antisense transcript expression. Michael E. Donaldson[1,2], H. Y. Kitty Cheung[1], Lauren A. Ostrowski[1], Kristi M. Goulet[1], Barry J. Saville[1,2]. 1) Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada K9J 7B8; 2) Forensic Science Program, Trent University, Peterborough, ON, Canada K9J 7B8.

Biotrophic fungal plant pathogens cause billions of dollars in losses to North American crops annually. The model for functional investigation of these fungi is *Ustilago maydis*. To expand our knowledge of this model and provide insight regarding the impact of RNA interference machinery loss on non-coding RNA profiles, comprehensive strand-specific RNA-seq was performed on cell-types of three related smut species: *U. maydis* (common smut of corn), S*porisorium reilianum* (head smut of corn), and *Ustilago hordei* (covered smut of barley) as well as a *U. maydis* SG200 growing *in planta* and *U. maydis - S. reilianum* 'hybrid' filamentous mycelia forced in culture. The resulting sequences were assembled into transfrags using Trinity, updated gene models were created using PASA and categorized with cuffcompare, and differential expression analysis was conducted using CLC Genomics Workbench. Transcript level variation was mapped onto the newly created comprehensive genome annotation, which included updated transcript structures for protein-coding genes and a large number of novel intergenic transcripts. Genes that were predicted for the first time with these RNA-seq analyses and genes with novel annotation features were independently assessed by reverse transcriptase PCR. Overall, the analyses revealed: 1) smut genomes encode a number of transcriptional units that is twice the number of annotated protein-coding genes, 2) some intergenic transcripts may encode proteins with characteristics of fungal effectors, 3) a large proportion of the identified antisense transcripts were detected at orthologous loci among the smut fungi, and 4) forced dikaryon formation between smut species differentially alters transcript representation from their respective 'parental' species. Functional investigation of the identified novel transcripts will provide new insight regarding the influence of antisense and intergenic transcripts on virulence in biotrophic fungal plant pathogens.

312. Comparative genomics of virulence towards malaria mosquitoes in *Beauveria bassiana*. Claudio Valero Jimenez[1,3], Daphne Spring in 't Veld[2], Sandra Smit[2], Constantianus J.M. Koenraadt[3], Bas J. Zwaan[1], Jan A.L. van Kan[4]. 1) Laboratory of Genetics, Wageningen University, Wageningen, Netherlands; 2) Bioinformatics, Wageningen University, Wageningen, Netherlands; 3) Laboratory of Entomology, Wageningen University, Wageningen, Netherlands; 4) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* have been proposed as biological control agents to kill malaria mosquitoes. Indeed, it has been shown that these fungi successfully reduce the lifespan of mosquitoes in the laboratory and in the field. Previously, we characterized the natural variation in virulence of 29 isolates of *Beauveria bassiana* and showed that there were up to 10-fold differences in virulence between the most virulent isolate compared to the least virulent isolate. This natural variation can be used to uncover the genetic mechanisms underpinning virulence, which will provide essential information for (i) further improving fungi as biocontrol agents, and (ii) estimating the likelihood of resistance development in the vector, i.e. mosquitoes. In this study, we sequenced 5 isolates representing the extremes of low/high virulence for further comparative genomic analysis. The genomes were *de novo* assembled and the draft genome size varied from 35.02 Mb to 38.83 Mb. The predicted encoding proteins were supported with three RNA-Seq libraries, and ranged from 10,283 to 10,831 genes. The core set consisted of 8800 genes shared between all isolates. We focused on the genome differences between isolates with contrasting virulence, with special emphasis on gene gain/loss, single nucleotide polymorphisms (SNPs), and secreted proteins. Our findings are discussed in the context of other sequenced entomopathogenic fungi (*Metarhizium anisopliae, M. robertsii* and *M. acridum*) as well as plant pathogenic fungi.

313. Comparative genomics of *Ashbya gossypii* and *Holleya sinecauda*.. George Greene, Fred Dietrich. Molecular Genetics and Microbiology, Duke University, Durham, NC.

Ashbya gossypii and *Holleya sinecauda* are related hemiascomycetes that differ in life cycle, with *A. gossypii* growing exclusively as a filamentous fungus, and *H. sinecauda* growing as a yeast that under nutrient stress can switch to hyphal growth. We are investigating gene differences between these organisms as a model system to investigate the difference between yeast and filamentous growth. Both organisms have small genomes of approximately 10kb with only around 4800 protein coding genes which simplifies this process.

These fungi are found in association with plant feeding insects, “true bugs” of the suborder Heteroptera. The fungi appear to have a symbiotic relationship, possibly involving providing nutrients, and in the case of *A. gossypii* riboflavin, to the host. We are also investigating the relationship between these fungi and insects.

Comparisons of these genomes with those of *Saccharomyces cerevisiae* show differences in the gene set that may be important adaptations for growth as a symbiont, and for filamentous growth.

314. The Phytophthora genus sequencing project. Brent Kronmiller[1], Danyu Shen[2], Javier Tabima[3], Stephanie Bollmann[4], Felipe Arredondo[3], Niklaus Grunwald[1,3,5], Brett Tyler[1,3], Beijing Genome Institute (BGI), Phytophthora Genus Sequencing Consortium. 1) Center for Genome Biology and Biocomputing, Oregon State University, Corvallis, OR; 2) Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China; 3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 4) Department of Integrative Biology, Oregon State University, Corvallis, OR; 5) Horticultural Crop Research Unit, USDA-ARS, Corvallis, OR.

Phytophthora is a genus of Oomycetes containing over 120 species that cause a huge variety of plant diseases. While some *Phytophthora* species infect only one plant host, some have a broad host range and can cause diseases on thousands of plants. Six species of *Phytophthora* have been previously sequenced. These six species are relatively diverse; they are found on four distinct clades of the ten-clade *Phytophthora* genus phylogenetic tree. Two sequenced species, *P. sojae* and *P. infestans*, have very different genomes. In order to better understand the differences observed within the *Phytophthora* genus, the Phytophthora Genus Sequencing Consortium has generated sequencing data for 29 additional species. Here we report on genomes from eleven *Phytophthora* species. On average 56M Illumina 90 base pairs (bp) paired end reads were sequenced per genome by the BGI. This resulted in approximately 5 Gb of sequence per genome - for a 100 Mb *Phytophthora* genome this equaled 50X coverage. Two RNA samples from each species were also sequenced to produce an average of 26M 90bp paired end transcript reads per genome. Genomes were assembled by the BGI. RNAseq reads were de-novo assembled to create transcriptomes for each species. Genes were annotated using the genomic and RNAseq data sets with MAKER, and functional annotations were assigned. Most species proved to be simple diploids. Concatenations of housekeeping protein sequences enable the phylogenetic relationships of the species to be accurately resolved within each clade. Effector proteins families showed large variations in number from species, through gene expansion and loss. A substantial number of effector families showed evidence of purifying selection within clades, occasionally with evidence of diversifying selection in one species.

315. Rapid Assembly and Functional Annotation of Diverse Fungal Genomes from Single Species and Metagenomic Populations Using Hi-C. Ivan Liachko, Joshua Burton, Jay Shendure, Maitreya Dunham. Genome Sciences, University of Washington, Seattle, WA.

Assembly of whole genomes from next-generation sequencing is inhibited by the lack of contiguity information in short-read sequencing. Assembly of whole genomes from mixed populations is currently impossible since one cannot tell which sequences originate from the same species within a population. This is especially true for eukaryotic genomes, such as fungi, which are usually excluded from metagenomic studies due to these technical limitations. We have overcome these bottlenecks by adapting a chromosome conformation capture technique (Hi-C) for deconvolution of metagenomes and assembly of single genomes.

To model the 3D structure of a genome, chromosome conformation capture techniques such as Hi-C are used to measure long-range interactions of DNA molecules. These tools employ crosslinking of chromatin in intact cells followed by intra-molecular ligation, joining DNA fragments that were physically nearby at the time of crosslink. Subsequent deep sequencing of these DNA junctions generates a genome-wide contact probability map that allows the reconstruction of high-quality genome assemblies and 3D modeling of genomic conformation within a cell. These methods also preserve the cellular origin of each DNA fragment and its interacting partner allowing for deconvolution of multi-chromosome genomes from a mixed population of organisms.

We have used Hi-C on mixed microbial populations to generate contact probability maps for over 25 diverse fungal species. These maps allow for comparative genomic study of chromosomal organization across species that fall in wide ranges of evolutionary distance. We have also been able to use this data to annotate functional features of genomes, such as centromeres. Additionally, our data has allowed us to produce improved genome assemblies for several sequenced yeasts by utilizing the enrichment of Hi-C signal at close intrachromosomal loci as an improved means of genome scaffolding.

We have applied our technology to diverse metagenomic populations such as craft beer, clinical infections, and tree endophyte samples to discover and assemble the genomes of novel strains of known species as well as previously unknown fungi.

316. Comparative genomics in Pezizomycetes: new insights into the evolution of ectomycorrhizal ascomycetes. T. Payen[1], C. Murat[1], A. Kohler[1], B. Henrissat[2], A. Kuo[3], B. Noel[4], P. Wincker[4], I. Grigoriev[3], F. Martin[1], Pezizomycete pan genome Consortium. 1) INRA, UMR1136, 54280 Champenoux, France; 2) UMR7257, Aix-Marseille Université, 13288 Marseille, France; 3) US DOE-JGI, Walnut Creek, CA 94598, United State; 4) Genoscope, 91057 Evry Cedex, France.

The Pezizomycetes comprise 200 genus and ~1,700 described species. They constitute an early diverging lineage of Ascomycota composed of saprophytic, mycorrhizal and pathogens species found in soils, wood decays, leaves, roots and dung. Some species are well known due to their edible fructifications, such as truffles and morels, and *Ascobolus immersus* is famous since the late 1930s as a model organism for genetic studies. Despite their interest, to date, only the genomes of *Tuber melanosporum* and *Pyronema confluens* have been published.

The aim of this study was to investigate genome evolution and gene repertoire idiosyncrasies in Pezizomycetes having different lifestyles (mycorrhizal versus saprotroph). Genomes of six Pezizomycetes were sequenced by the Joint Genome Institute and the Genoscope Genome Institute. Genome sizes showed important variation, from 48 Mbp in *Morchella conica* to 192 Mbp in *Tuber magnatum*. The number of protein-coding genes is also highly variable in the pezizomycetes ranging from ~ 9,000 for *Tuber* spp to ~17,000 for *Choiromyces venosus*. Ectomycorrhizal (ECM) pezizomycetes (e.g., *Tuber* spp) have a reduced complement of genes encoding plant cell wall degrading enzymes (PCWDE), compared to saprotrophic pezizomycetes (e.g. *A. immersus*). Nevertheless, *Tuber* species have retained a unique array of PCWDE, including a GH6 cellulase, suggesting they may possess limited ligninolytic capabilities. The loss of most of the glycoside hydrolases (GHs) acting on plant cell walls is shared with ECM basidiomycetes. Polyphyletic evolution of the ECM lifestyle is thus marked by convergent losses of different components of the ancestral saprotrophic apparatus. This study provides new insights in ECM symbiosis evolution, highlighting divergence and convergence between Basidiomycota and Ascomycota symbiotic species.

317. Comparative genomics of Leotiomycetes suggests a high saprotrophic potential irrespective of fungal ecological strategies. E. Martino[1], A. Kuo[4], G. Grelet[3], S. Daghino[1], A. Kohler[2], E. Morin[2], C. Murat[2], S. Casarrubia[1], H-R. Khouja[1], B. Henrissat[5], C. Veneault-Fourrey[2], IV. Grigoriev[4], S. Perotto[1], F. Martin[2]. 1) DBIOS, University of Turin, 10125 Turin, Italy; 2) INRA, UMR 1136, Lab of Excellence ARBRE, 54280 Champenoux, France; 3) Landcare Research, Lincoln 7640, New Zealand; 4) US DOE-JGI, Walnut Creek, CA 94598, USA; 5) UMR 725, Aix-Marseille Université, 13288 Marseille, France.

Fungi in the Leotiomycetes are ecologically diverse and colonize a variety of habitats as saprotrophs, pathogens or mutualists. Mycorrhizal Leotiomycetes include ericoid endomycorrhizal (ERM) fungi, and the genomes of four ERM fungi (*Oidiodendron maius, Meliniomyces bicolor, M. variabilis, Rhizoscyphus ericae*) were sequenced and compared with seven other sequenced Leotiomycetes displaying different ecological strategies (endophytes, saprophytes, plant pathogens). Large variations in genome size (28 Mbp for *Amorphotheca resinae* to 118 Mbp for *Blumeria graminis*) and number of protein-coding genes (6,470 for *B. graminis* to 22,766 for *Cadophora* sp) were observed. A peculiar feature of these 11 Leotiomycetes was their large complement of genes encoding plant cell wall degrading enzymes (PCWDEs). Amongst 61 taxonomically diverse fungi with different ecological strategies, 5 Leotiomycetes (3 of them being ERM fungi) were in the top 10 CAZymes richest fungi. Analyses of specific CAZymes families showed that, if compared to ectomycorrhizal fungi, ERM fungi maintain multiple copies of GH6, GH7 and LPMO lytic polysaccharide monooxygenases (GH61/AA9) genes. These findings suggest that a tight control of CAZymes takes place during the symbiotic interaction. RNA-Seq was thus used to further investigate expression of CAZymes in the ERM symbiosis. Among the symbiosis regulated genes identified in *O. maius* were several CAZymes, transporters, oxidoreductases and SSPs. *O. maius* expressed a large set of PCWDEs in symbiosis, 28% of *O. maius* CAZymes being upregulated in mycorrhizal roots. These symbiosis-regulated CAZymes may be used to penetrate the thick host cell wall during root colonization, but they may also facilitate nutrient mobilisation from organic substrates, such as *Sphagnum* peat, thus supplementing host plant photosynthesis with fungal-derived carbon.

318. Characterizing chromosomal, inter and intragenic variation with whole genome re-sequencing of *Zymoseptoria tritici*. Megan McDonald[1], Andrew Milgate[2], James Hane[3], Angela Williams[3], Peter Solomon[1]. 1) Plant Science, Research School of Biology, The Australian National University, Canberra, ACT, Australia; 2) NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia; 3) Centre for Crop and Disease Management, Curtin University, Perth, WA, Australia.

Zymoseptoria tritici is a necrotrophic pathogen of wheat, with an extended latent period, followed by necrosis and active pathogen growth. The mechanisms through which the fungus rapidly induces host cell death remain largely unknown and is currently hypothesized to be mediated by the secretion of small proteins or metabolites. These molecules are commonly referred to in fungal pathogens as effectors. This work seeks to exploit variation at the genomic level on a differential set of 13 isolates known to vary in virulence towards a range of Australian wheat cultivars in order to identify effector gene candidates. We use whole genome re-sequencing to identify probable presence/absence polymorphisms in both whole chromosomes and genes across our differential isolates. *In silico* gene presence/absence calling was performed using two independent methods. One method used a sliding window approach that examined mapped read coverage to the reference genome, requiring a minimum of 10X coverage. Separately, *de novo* assemblies of each re-sequenced genome were probed for the presence of each predicted gene using BLASTN, at least 50% of the total gene length must be found in the top BLAST hit to be considered present. Absences of each gene were called if both independent methods agreed a gene was absent as well as the gene occurred in the region of the genome where NGS reads can be mapped reliably. This analysis shows much higher gene loss on the accessory chromosomes when compared to core chromosomes. We also quantified non-synonymous (NS) variation between differential isolates, separating NS single nucleotide polymorphisms into RIP associated or non-RIP associated categories. Though closely related, we show a small subset of genes that have been variably affected by RIP in the 13 pathotypes. In conjunction with publically available expression data (from RNA-seq), we have generated a short list of 56 variable genes for functional validation through genetic knock-outs.

319. Genome-wide annotation and evolutionary analysis of cytochrome P450 monooxygenases in the conifer pathogen *Heterobasidion irregulare*. Anthony C Mgbeahuruike[1], Andriy Kovalchuk[2], Wimal Ubhayasekera[3], David R. Nelson[4], Jagjit. S. Yadav[5]. 1) Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Enugu State, Nigeria; 2) Department of Forest Sciences, University of Helsinki, FI00014, Helsinki, Finland; 3) Department of Molecular Biology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; 4) Department of Microbiology, Immunology, and Biochemistry, University of Tennessee, Memphis, TN 38163, USA; 5) Environmental Genetics and Molecular Toxicology Division, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0056, USA.

The conifer pathogen, *Heterobasidion annosum* s. l. has a large repertoire of P450s (P450ome) but the functional diversity and evolutionary mechanisms driving these hemeproteins are not known. A genome-wide structural and evolutionary analysis of P450s in *H. irregulare* was carried out. Using the International P450 Nomenclature criteria, *H. irregulare* P450ome was classified into 11 clans, 35 families and 64 subfamilies. Cluster analysis of the P450ome showed presence of multigene families organized in clusters of tandem repeats. The largest of the clusters was found on scaffold 5 in subfamily M of family CYP5144. The identified clusters consist of closely related genes belonging to the same family and subfamily, an indication that the identified clusters may have originated from recent gene duplication events. Some of the identified clusters included putative pseudogenes. The pseudogenization could be an outcome of gene duplication, as both copies originating from the duplication event are identical and functionally redundant. Phylogenetic analysis showed that all the clans and families of *H. irregulare* P450ome were monophyletic groups except family CYP5144, the largest family of *H. irregulare* P450ome that appeared as polyphyletic group. Homology modeling identified conserved residues in the catalytic sites of most of the analysed gene families including several of the conserved heme-binding residues. The high level of P450 gene diversity may have evolved by extensive gene duplications probably caused by the high metabolic demands of this group of fungi in their ecological niches.

320. Genomic analyses of biotrophic plant-pathogenic protists in the Rhizaria kingdom. Arne Schwelm[1], Johan Fogelqvist[1], Jutta Ludwig-Müller[2], Christina Dixelius[1]. 1) Swedish University of Agricultural Sciences, Department of Plant Biology, Box 7080, 75007 Uppsala, SwedenDepartment of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Institute of Botany, Technische Universität Dresden, 01062 Dresden, Germany.

Plasmodiophora brassicae is a major disease threat for Brassica oil and vegetable crop production worldwide. The causal agent is a Plasmodiophorid, which are obligate biotrophic plant-pathogenic protists in the Rhizarian kingdom. Although the Plasmodiophorids include other important agricultural pathogens such as *Polymyxa betae*, *Spongospora subterranea*, their biology remains poorly understood due to their intracellular biotrophic life style. Here we present the 25.5 Mb genome sequence with 9,730 gene models of *P. brassicae,* developmental stage-specific transcriptomes and a transcriptome of the related species *Spongospora subterranea.* We provide the first genomic data for pathogenic Rhizaria. Like other biotrophic pathogens both Plasmodiophorids show a reduction in metabolic pathways. We report proteins that can modify the plant hormones, effector candidates and analyses of carbohydrate active enzymes, which differ from plant pathogenic Oomycetes and fungi. The exploitation of the life stage specific transcripts will shed light in the understanding of the life cycle at a molecular basis, which will in the long run help to understand and control club root disease. Our data also fill an important gap for the understanding of the eukaryotic tree of life, since we provide only the third genome of the Rhizarian kingdom.

321. The occurrence of chromosomal rearrangements in the fungal genus *Verticillium*. Xiaoqian Shi[1], Melvin D. Bolton[2], Michael Seidl[1], Luigi Faino[1], Bart P.H.J. Thomma[1]. 1) Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; 2) Northern Crop Science Laboratory, USDA, Fargo, ND, USA.

Based on a recent comparative population genomics study, extensive chromosomal rearrangements between strains of the plant pathogenic species *V. dahliae have been found (de Jonge et al., 2013)*. The rearrangements result in the occurrence of lineage-specific genomic regions that appear to be greatly enriched for *in planta*-expressed genes that encode virulence factors that enable host colonization. Thus, it is speculated that genomic rearrangements foster evolution of aggressiveness in the asexual pathogen *V. dahliae* (de Jonge et al., 2013). In this project, we aim to investigate the occurrence and regulation of chromosomal rearrangements in the genus *Verticillium* that comprises plant pathogenic (*V. dahliae, V. longisporum, V. albo-atrum, V. alfafae, V. nonalfalfae*), and saprophytic and weakly pathogenic (*V. tricorpus, V. zagamsianum, V. nubilum, V. isaacii and V. klebahnii*) species. We hypothesize that chromosomal rearrangements occur in the virulent plant pathogens and are not observed in the weak pathogens and saprophytes. To investigate this hypothesis, the genomes of multiple strains of each of the species within the *Verticillium* genus were sequenced and assembled to investigate chromosomal structures within and between each of the species. Furthermore, we investigated chromosomal size polymorphisms based on karyotyping. Collectively, our data show that inter-chromosomal rearrangements are not confined to pathogenic *Verticillium* spp.

322. Comparative genomics of the Sigatoka disease complex on banana. Ti-Cheng Chang, Ioannis Stergiopoulos. Department of Plant Pathology, University of California Davis, One Shields Avenue, Davis, California 95616, USA.

Banana is among the world's five most significant staple food crops, grown extensively throughout the tropics and subtropics. However, bananas are prone to many diseases that reduce production and have a negative socio-economic impact, especially in developing countries and communities that depend on this crop for their survival. Currently, the Sigatoka disease complex caused by the related Dothideomycete fungi *Mycosphaerella fijiensis* (black sigatoka), *Mycosphaerella musicola* (yellow sigatoka), and *Mycosphaerella eumusae (*eumusae leaf spot) is the most devastating disease on banana worldwide, reducing yields by more than 38%. The three species have emerged on bananas during the last century assumingly from a common ancestral species and, although they share a similar hemibiotrophic lifestyle and disease-cycle on their banana host, clear differences in virulence exist among them. In order to understand the evolutionary trends and genomic modifications associated with shifts in their virulence spectra and to identify their pathogenic core that can be exploited in disease management programs, we have sequenced and analyzed the genomes of *M. eumusae* and *M. musicola* and compared them with the available genome sequence of *M. fijiensis*. Despite their close phylogenetic relatedness, the three species differ in their genome sizes,

mainly due to different rates of LTR retrotransposon proliferation. Still, gene counts are relatively the same and in the range of other Dothideomycete species. Analyses of gene content showed that ~13% of the genes in each species are species-specific, suggesting that they participate in species-specific processes. 234 gene families were also identified, including 18 secreted protein families and 8 putative effectors that were shared specifically by the three species and no other fungi, and which could thus be related to adaptation to the banana host. Notably, only 19 effectors (~15%) were shared among the three species, suggesting that altered effector repertoires partially forms the basis of their differential virulence. An excess of positive selection on putative effectors and the secretome in general as compared to non-secreted proteins was also evident, further reinforcing this notion.

323. MADS-box transcription factors as master regulators of yeast-to-mycelium transition in *Penicillium marneffei*. Ence Yang[1], Gang Wang[1], Xiaorong Lin[2], James Cai[1]. 1) Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX; 2) Department of Biology, Texas A&M University, College Station, TX.

Penicillium marneffei is a systemic dimorphic fungus growing in an infectious mold form in the soil and a pathogenic yeast form in mammalian hosts. The temperature-dependent dimorphic transition between mycelium and yeast is considered crucial for its pathogenicity and transmission. However, the underlying mechanisms are still poorly understood. Here, we united various high-throughput genomic technologies to pinpoint the master transcription factors that regulate the dimorphic transition. Specifically, we performed a hybrid assembly of *P. marneffei* strain PM1 using sequences generated with multiple sequencing platforms, and determined gene expression levels using RNA sequencing in cells at the mycelial and yeast phases, as well as during phase transition. We found that a group of MADS-box transcription factor genes, whose family is exclusively expanded in *P. marneffei*, were differentially and selectively expressed during transition. Special attention was paid to one of these genes, *madsA*, which is located within the genomic loci composed of a cluster of genes highly expressed during yeast-to-mycelium transition. Overexpression of *madsA* induced the yeast-to-mycelium transition at restrictive temperature 37°C, while knockdown of *madsA* delayed the yeast-to-mycelium transition at 25°C. Our findings suggested that madsA was a master regulator of yeast-to-mycelium transition in *P. marneffei*.

Education and Professional Development

324. Investing in the Fungal Genetics Stock Center: Kansas State Plant Pathology offers new home for collection. Kevin McCluskey, John Leslie. Fungal Genetics Stock Center, Department of Plant Pathology, Kansas State University, Manhattan, KS.

In response to changes in federal support for living collections, the Fungal Genetics Stock Center relocated in November 2014 to the department of Plant Pathology at Kansas State University. The relocation included over thirteen thousand pounds of equipment and materials and was accomplished in one day. Despite this significant disruption, the collection continued to provide high quality resources with very few delays.

With over 25,000 accessioned fungal strains including over 13,000 gene deletion and molecularly modified (GMO) strains, the FGSC is the largest collection of genetically characterized and manipulated fungal strains in the world. In addition to these accessioned strains, the FGSC holds 775 plasmids and nearly 5,000 deletion mutants for Cryptococcus or Candida contributing to its global leadership in curating and distributing materials to researchers around the world. Existing collections at Kansas State complement the FGSC holdings and emphasize the synergy of bringing the FGSC to KSU.

Gene Regulation

325. Interconnection of tryptophan with secondary metabolism in *Aspergillus fumigatus*. Tsokyi Choera[1], Pinmei Wang[1], Philipp Wiemann[1], Tippapha Pisithkul[2], Daniel Amador-Noguez[2], Nancy P. Keller[1,2]. 1) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 2) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Small peptides formed from non-ribosomal peptide synthetases (NRPS) are bioactive molecules produced by many fungi including the genus *Aspergillus.* A subset of NRPS utilize tryptophan and the non-proteogenic amino acid anthranilate in synthesis of various metabolites such as *A. fumigatus* fumiquinazolines (Fqs). Here we examine the contribution of both anabolic and catabolic tryptophan pathways to Fq synthesis. Anthranilate synthase, TrpE, a key enzyme in the synthesis of anthranilate, which is then converted to tryptophan, contains a conserved negative feedback domain responsive to tryptophan levels. Site specific mutations of this domain results in increased synthesis of anthranilate and FqC under tryptophan insufficiency. We also explore the contributions of tryptophan degradative pathways to Fq synthesis.

326. Regulation of SrbA in *Aspergillus fumigatus*. Sourabh Dhingra[1], Dawoon Chung[1], Özgür Bayram[2], Jay C. Buckey[3], Robert A. Cramer[1]. 1) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Biology, Maynooth University, National University of Ireland, Kildare, Ireland; 3) Department of Medicine, Geisel School of Medicine at Dartmouth, Lebanon, NH.

Aspergillus fumigatus (Afum) is the primary causal agent of invasive aspergillosis. In the infection site microenvironment, Afum encounters and adapts to hypoxia, a necessary virulence trait. The sterol regulatory element binding proteins (SREBPs), SrbA and SrbB, are necessary for hypoxia adaptation and virulence, however, mechanisms of SrbA/SrbB regulation remain to be elucidated. Importantly, Afum like other Eurotiomycetes appears to lack the sterol sensing protein SCAP critical for SREBP regulation in other eukaryotes. A golgi E3 ubiquitin ligase complex encoded by the *dsc* genes is critical for proteolytic cleavage and regulation of SrbA. Yet, the mechanisms by which this cleavage event is regulated remain to be determined. Proteins that directly interact with SrbA are candidates for potential novel regulatory mechanisms.To identify SrbA interacting proteins, we performed GFP-trap pull-down experiments with GFP attached to either

the N terminus or C terminus of SrbA and constitutively active SrbA. Our results identified different classes of proteins bound to the N terminus and C terminus of SrbA, suggesting potential layers of regulatory mechanisms. To identify upstream genetic regulatory elements of SrbA activation, we are also initiating protein pull downs with DscA::S-tag as the bait protein.As the SrbA genetic network responds to hypoxia and is critical for virulence, we are also testing the effects of alleviating hypoxia at the infection site by using hyperbaric oxygen (HBO). Our preliminary results show HBO therapy (HBOT) can reduce the rate of fungal germination and metabolism *in vitro,* in part through inhibition of the SrbA genetic network. In a murine model of IPA HBOT reduced fungal burden and increased survival. Taken together, our data suggest that manipulation of infection site oxygen availability can alter the physiology of the invading fungus in part through manipulation of a major fungal oxygen sensing pathway.

327. Regulating Nonribosomal Peptide Synthesis: Post-biosynthetic bis-Thiomethylation of Gliotoxin Attenuates Gliotoxin Biosynthesis in *Aspergillus fumigatus*. Stephen Dolan, Rebecca Owens, Grainne O'Keeffe, Gary Jones, Sean Doyle. Department of Biology, Maynooth University, Kildare, Ireland.

Gliotoxin (GT) is a redox-active nonribosomal peptide produced by *Aspergillus fumigatus*. Like many other disulfide-containing epipolythiodioxopiperazines, a bis-thiomethylated form is also produced. In the case of GT, bisdethiobis(methylthio)gliotoxin (BmGT) is formed for unknown reasons by a cryptic enzyme. Here, we identify the S-adenosylmethionine dependent gliotoxin bis-thiomethyltransferase (GtmA), which converts dithiol GT to BmGT.

Disruption of this previously unclassified non-*gli* cluster encoded methyltransferase completely abrogated organismal ability to biosynthesize and secrete BmGT, while GT production and secretion were increased (p=0.0056). Surprisingly, exposure of *A. fumigatus ΔgtmA* to exogenous GT did not reveal the acquisition of a sensitive phenotype compared to the wild-type strain. Thus, GtmA-mediated GT bismethylation is not essential for self-protection against GT. The activity of GtmA, which is induced by exogenous GT, is only detectable in protein lysates of *A. fumigatus* deficient in the gliotoxin oxidoreductase, *gliT*. Recombinant GtmA was shown to bismethylate dithiol GT using S-adenosyl methionine as methyl donor, via a novel LC-MS enabled activity assay. GtmA is the first characterised methyltransferase capable of substrate bis-thiomethylation in any organism. Label-free quantitative (LFQ) proteomics of *A. fumigatus* wild-type, *ΔgtmA*, and *gtmAC* strains cultured in Czapek-Dox media revealed an elevated abundance of *gli* cluster encoded enzymes in the *ΔgtmA* mutant strain. Also, LFQ proteomics revealed that exogenously added GT and BmGT induce differential remodelling of the *A. fumigatus* proteome. Phylogenetic analysis of this enzyme revealed that there are 124 GtmA homologs within the Ascomycota phylum.

We now propose that the purpose of GtmA mediated BmGT formation primarily serves to attenuate GT biosynthesis. This appears to be the first example of postbiosynthetic regulation of nonribosomal peptide synthesis in any organism.

328. PpoA coordinates fungal development through phialide formation and regulation of secondary metabolite clusters in *Aspergillus fumigatus*. Gregory Fischer[1], Erwin Berthier[2], Chun-Jun Guo[3], Jonathan Palmer[2], Clay C.C. Wang[3], Nancy Keller[2,1]. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Pharmacology and Pharmaceutical Sciences and Chemistry, University of Southern California, Los Angeles, CA.

The opportunistic pathogen *Aspergillus fumigatus* contains three cyclooxygenase (COX)-like enzymes termed PpoA, PpoB, and PpoC (**P**si-factor **p**roducing **o**xygenase). Ppos produce bioactive oxygenated lipid signaling molecules (oxylipins) important for fungal development and similar in structure to mammalian prostaglandins. Here, we identified that disruption of PpoA accelerates asexual development, shifts secondary metabolite production, and significantly disrupts phialide development. PpoA loss and overexpression (OE) increased and delayed spore development, respectively. Northern blot and biochemical analysis of wild-type, *ΔppoA,* and *OE::ppoA* strains indicate a major shift in secondary metabolism including production of several spore specific metabolites such as DHN melanin and endocrocin. Furthermore, deletion of PpoA resulted in long, filamentous phialides protruding from the vesicle of the conidiophore. The abnormal phialide phenotype could be rescued by co-culture of the *ΔppoA* strain with the *OE::ppoA* strain. This work demonstrates the critical role PpoA-derived oxylipins play in *A. fumigatus* development and future work will attempt to identify the mechanism by which PpoA affects phialide formation.

329. A copper-responsive secondary metabolite pathway in *Aspergillus fumigatus*. Fang Yun Lim[1], Joshua A. Baccile[2], Frank C. Schroeder[2], Nancy P. Keller[1]. 1) Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 2) Boyce Thompson Institute Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

Copper is a redox-active transition metal indispensible for life. It serves as a cofactor to enzymes involved in various fundamental cellular processes (e.g. respiration, reductive iron uptake, superoxide detoxification, etc.). In pathogenic fungi, copper is required for the function of virulence modulators such as multicopper laccases and superoxide dismutases. Here we report on the identification of a copper-responsive secondary metabolite cluster (*crm*) in the human opportunistic pathogen *Aspergillus fumigatus*. Bioinformatic prediction coupled with expression analyses suggests that the current *crm* cluster spans a minimal of five enzymatic genes. Preliminary results show that expression of the *crm* cluster is enhanced during vegetative growth and under copper-deficient condition. Furthermore, our preliminary observations suggest a potential crosstalk between the *crm* cluster and the recently identified iron-responsive *has* cluster in this species. Future studies will elucidate the potential role of the *crm* pathway product(s) in copper-uptake, the nature of this crosstalk between two copper- and iron-responsive secondary metabolite clusters, and potential role in the biology and pathogenicity of this fungus.

330. The *pkcA* gene is required for the heat adaptation in *Aspergillus fumigatus*. Marina Campos Rocha, Krissia Franco de Godoy, Anderson Ferreira da Cunha, Iran Malavazi. Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil.

The CWIP (cell wall integrity pathway) signaling cascade is activated in fungal cells under stressing conditions and plays a role in the adaptation of several fungal pathogens to the human host. We have shown that *A. fumigatus pkcA* is involved in the CWIP since a point mutation in the *pkcA* gene (*pkcA*^{G579R}) located in the cysteine-rich C1B regulatory domain leads to defective activation of the MAP kinase MpkA and several cell-wall related phenotypes. Here we show that the *pkcA* loss-of-function is associated with impaired thermotolerant growth in *A. fumigatus*. Polar and vegetative growth of the *pkcA*^{G579R} mutant strain was affected both at 37ºC and 45ºC and could be partially restored by D-sorbitol. Conidia survival in the mutant strain was also reduced about 50% when exposed to 45ºC and 50ºC in comparison to the wild type strain. Since Hsp90 has been shown to orchestrate cellular signaling that governs drug resistance and developmental transitions in other fungal pathogens, we wanted to investigate a possible interaction of Hsp90 and PkcA using the Hsp90 pharmacological inhibitor radicicol. The mutant strain *pkcA*^{G579R} was more sensitive to radicicol indicating a role of Hsp90 in the function of PkcA. The transcriptional basis of the *pkcA* involvement in heat adaptation was also evaluated by analyzing the expression of the genes known to be involved in heat shock response in *S. cerevisiae* or *A. fumigatus* such as *hsf1*, *hsp30*, *and hsp90*, *ssc1* (a mitochondrial Hsp70 chaperone). Notably, the expression of *pkcA* is increased both at 37ºC and 48ºC indicating that the expression of *pkcA* is part of the cell response to heat adaptation. On the other hand, lower levels of expression were observed for the heat sock-related genes in the mutant background. These results show that the defective function of the CWIP in *A. fumigatus* causes alteration in the heat shock response and suggests an interaction between PkcA Hsp90 molecular chaperone in *A. fumigatus.*

331. The putative RNA Polymerase II transcription elongation factor-like protein, *rtfA*, regulates growth, conidiation, and pathogenesis in *Aspergillus fumigatus*.. Ryan Myers, Timothy Smith, Ana Calvo. Northern Illinois University, Dekalb, IL.

Invasive aspergillosis by *Aspergillus fumigatus* is a leading cause of infection-related mortality in immune-compromised patients. This population group includes individuals infected with HIV, cancer patients undergoing chemotherapy, organ transplant patients, individuals with genetic immune deficiencies, and those with hematological malignancies. The number of patients who fall into these categories is steadily increasing. In order to discover potential genetic targets to control *A. fumigatus* infections, we have characterized *rtfA*, a gene encoding a conserved putative RNA polymerase II transcription elongation factor-like protein. Recently, work from our laboratory has shown that the *rtfA* ortholog in the model fungus *Aspergillus nidulans* influences morphogenesis and secondary metabolism. In the present study we are investigating the regulatory output of *rtfA* in *A. fumigatus*. Our results revealed that *rftA* influences fungal growth and conidiation, as well as protease activity and response to oxidative stress in this opportunistic human pathogen. Furthermore, *rtfA* was shown to be a virulence factor in the *Galleria Mellonella* infection model system.

332. AmcA - a mitochondrial ornithine transporter involved in fungal siderophore biosynthesis. Lukas Schafferer[1], Nicola Beckmann[1], Ulrike Binder[2], Hubertus Haas[1]. 1) Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria; 2) Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria.

Iron is an essential nutrient required for a wide range of cellular processes. The opportunistic fungal pathogen *Aspergillus fumigatus* employs low-molecular mass iron-specific chelators, termed siderophores, for uptake, storage and intracellular iron distribution, which play a crucial role in the pathogenicity of this fungus. Siderophore biosynthesis depends on coordination with the supply of its precursor ornithine, produced mitochondrially from glutamate or cytosolically via hydrolysis of arginine. In this study, we demonstrate a role of the mitochondrial transporter AmcA (AFUA_8g02760) in siderophore biosynthesis by *A. fumigatus.*

Consistent with a role in mitochondrial ornithine export, AmcA-deficiency resulted in decreased cellular ornithine and arginine contents as well as decreased siderophore production on glutamine as the sole nitrogen source. In support, arginine and ornithine as nitrogen sources did not impact siderophore biosynthesis due to cytosolic ornithine availability. As revealed by Northern blot analysis, transcript levels of siderophore biosynthetic genes were unresponsive to the cellular ornithine level. In contrast to siderophore production, AmcA deficiency did not alter the cellular content of polyamines, demonstrating cellular prioritization of ornithine use. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to the polyamine biosynthesis inhibitor eflornithine, most likely due to the decreased ornithine pool. AmcA-deficiency decreased the growth rate particularly on ornithine as the sole nitrogen source during iron starvation and sufficiency, indicating an additional role in the metabolism and fitness of *A. fumigatus*, possibly in mitochondrial ornithine import. In the *Galleria mellonella* larvae infection model, AmcA-deficiency did not affect virulence of *A. fumigatus*, most likely due to the residual siderophore production and arginine availability in the host niches.

333. Regulation of copper homeostasis in *Aspergillus fumigatus*. Philipp Wiemann[1], Joshua A. Baccile[2], Frank C. Schroeder[2], Nancy P. Keller[1]. 1) Medical Microbiology & Immunology, University of Wisconsin-Madison, Madison, WI; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

Regulation of metal acquisition and detoxification plays a critical role in survival and virulence of the opportunistic pathogen *Aspergillus fumigatus*. Macrophages phagocytizing *A. fumigatus* spores are thought to employ two major strategies for killing the pathogen, 1. Acidification and export of reduced Fe^{2+} and 2. Production of superoxide by the NADPH oxidase (NOX) complex and influx of Cu^{+} ions. Regulation of iron homeostasis is best understood in *A. fumigatus* whereas very little is known about copper-dependent regulation. We recently demonstrated that the non-ribosomal peptide, hexadehydroastechrome (HAS; a tryptophan-derived iron (III)-complex) is involved in *A. fumigatus* virulence and iron homeostasis[1,2]. Recently we found that HAS also influences genes involved in copper acquisition and detoxification. Here we present our latest results on copper binding of HAS and regulation of copper-dependent genes mediated by homologs of the *Saccharomyces cerevisiae* transcription factors Mac1/Cuf1 and Ace1/Cup2 in *A. fumigatus*.

[1]Yin *et al*., (2013) *J Am Chem Soc* **135**:2064-2067.

[2]Wiemann *et al*., (2014) *Front Microbiol* **5**:530.

334. Fungal artificial chromosomes for mining of the fungal secondary metabolome. J. Bok[1], J. Albright[2], R. Ye[3,4], D. Mead[4], M. Wagner[4], A. Krerowicz[4], A. Goering[5], K. Clevenger[5], T. Velk[1], P. Thomas[5], N. Kelleher[2,5], N. Keller[1], C. Wu[3,4]. 1) Department of Medical Microbiology & Immunology, University of Wisconsin, Madison, Wisconsin, USA; 2) Department of Chemistry, Northwestern University, Evanston, Illinois, USA; 3) Intact Genomics, Inc. St Louis, Missouri, USA; 4) Lucigen Corporation, Middleton, Wisconsin, USA; 5) Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois, USA.

With thousands of fungal genomes being sequenced, each genome containing up to 70 secondary metabolite (SM) clusters 30 - 80 kb in size, breakthrough techniques are needed to characterize this SM wealth. Here we describe a novel system-level methodology for unbiased cloning of intact SM clusters from a single fungal genome for one-step transformation and expression in a model host. All 56 intact SM clusters from *Aspergillus terreus* were individually captured in self-replicating fungal artificial chromosomes (FACs) containing both *E. coli* F replicon and an *Aspergillus* autonomously replicating sequence (AMA1). Candidate FACs were successfully shuttled between *E. coli* and the heterologous expression host *A. nidulans*. As proof-of-concept, an *A. nidulans* FAC strain was characterized in a novel liquid chromatography-high resolution mass spectrometry (LC-HRMS) and data analysis pipeline leading to the discovery of the *A. terreus* astechrome machinery.

335. The putative polysaccharide synthase gene *cpsA* regulates mycotoxin production and morphogenesis in the fungus *Aspergillus nidulans*. Xuehuan Feng[1], Vellaisamy Ramamoorthy[2], Ana Calvo[1]. 1) Department of Biological Sciences, Northern Illinois University, 155 Castle Drive, DeKalb, Illinois 60115, USA; 2) Department of Plant Biotechnology, Center for Plant Molecular Biology (CPMB), Tamil Nadu Agricultural University, Coimbatore - 641003, India.

The model filamentous fungus *Aspergillus nidulans* synthesizes a variety of secondary metabolites, such as the mycotoxin sterigmatocystin (ST). The production of this toxin is positively controlled by *veA*, a global regulatory protein that also governs sexual and asexual development in *A. nidulans*. In the absence of *veA*, the biosynthesis of ST is blocked. We performed random mutagenesis in a deletion *veA* strain and identified several revertant mutants that are able to synthesize ST, among them RM1. This mutant is also strongly defective in asexual and sexual morphogenesis. Complementation of RM1 with a genomic library revealed that the mutation occurred in the coding region of a gene that encodes a putative polysaccharide synthase designated as *cpsA*. In a *veA* wild-type background, deletion of *cpsA* delays production of ST, and shows a reduction of conidial production and defective sexual stage. These results indicate that *cpsA* is important for mycotoxin biosynthesis and it is involved in *A. nidulans* morphogenesis. In addition, the deletion of *cpsA* results in a strain more sensitive to oxidative stress and reduces its ability to form biofilm, suggesting that *cpsA* might contribute to protecting fungal colonies from environmental stresses.

336. Distinct histone amino acids are involved in controlling secondary metabolism in *Aspergillus nidulans*. Juliane Fischer[1,3], Hans-Wilhem Nützmann[1], Kirstin Scherlach[2], Christian Hertweck[2], Axel A. Brakhage[1,3]. 1) Departments of Molecular and Applied Microbiology, Hans-Knöll-Institut, Jena, Germany; 2) Biomolecular Chemistry, Hans-Knöll-Institut, Jena, Germany; 3) Friedrich Schiller University Jena, Jena, Germany.

Chromatin remodeling events have recently been discovered to play an important role in the secondary metabolism of filamentous fungi (1). Previously, we showed that a bacterium, *Streptomyces rapamycinicus*, is able to re-program the histone modifying SAGA/Ada complex including the histone acetyltransferase GcnE of the important model fungus *Apergillus nidulans* (2, 3). As a consequence, lysine 9 and lysine 14 of distinct secondary metabolism genes were specifically acetylated during the bacterial fungal interaction, which, furthermore, led to the activation of the silent orsellinic acid gene cluster. To prove the importance of histone modifications for distinct gene expression profiles, we exchanged several amino acids of histone H3 of *A. nidulans* (4). These amino acids included lysine residues 9, 14, 18, and 23 as well as serine 10 and threonine 11. Lysine residues and serine/ threonine were exchanged to arginine and alanine respectively. Interestingly, all the strains produced were viable, allowing to analyse directly the consequences of missing posttranslational histone modifications. In the mutant strains, for the penicillin, sterigmatocystin and orsellinic acid biosyntheses, we detected major changes of both the expression patterns at the transcriptional level and the metabolite level of the respective gene clusters. These effects were mainly due to lysine 9 and lysine 14 of histone H3. Taken together, for the first time we show a causal linkage between the lack of acetylation of lysine residues on histone H3 and the transcription and product formation of important secondary metabolites. This leads to the question how the SAGA/ADA-dependent histone acetylation program is specifically targeted to a distinct gene cluster on the genome and how this is induced by a bacterium. (1) Brakhage (2013) *Nat. Rev. Microbiol*., (2) Schroeckh *et al*. (2009) *PNAS*, (3) Nützmann *et al*. (2011) *PNAS*, (4) Nützmann, Fischer *et al.* (2013) *AEM*.

337. Regulation of Nuclear Import of the *Aspergillus nidulans* GATA Transcription Factor AreA. Cameron Hunter[1], Margaret Katz[2], Richard Todd[1]. 1) Plant Pathology, Kansas State University, Manhattan, KS; 2) Molecular and Cellular Biology, University of New England, Armidale, NSW.

The TOR signaling pathway is conserved throughout eukaryotes and coordinates cell growth in response to nutrient availability. In *Saccharomyces cerevisiae* the TOR pathway is inhibited by the anti-tumorigenic immunosuppressant, rapamycin. This leads to downstream effects, including nuclear localization of the GATA transcription factors Gln3p and Gat1p, which activate nitrogen metabolic genes. The components of the TOR pathway are conserved between yeast and *Aspergillus nidulans.* We have used an epitope tagged version of AreA to investigate the effects of rapamycin and the TOR pathway on AreA subcellular localization. In stark contrast to triggering nuclear import of Gln3p and Gat1p in yeast, rapamycin does not cause nuclear localization of AreA. Instead, rapamycin prevents AreA nuclear accumulation in response to nitrogen starvation. Nuclear accumulated AreA is rapidly exported from the nucleus in response to nitrogen

nutrients or carbon starvation, but addition of rapamycin to nitrogen-starved cells does not trigger AreA nuclear export, suggesting that the TOR pathway controls AreA nuclear import. We are investigating the effects on AreA nuclear localization of the other components in the TOR pathway SitA, JipA, and GstA. In yeast, deletion of the cytoplasmic anchor Ure2p confers Gln3p nuclear localization. In contrast, deletion of the Ure2p homolog GstA prevents AreA nuclear accumulation. We also show that AreA does not accumulate in the nucleus in a loss of function mutant affecting the carbon starvation and autolysis transcription factor XprG. Collectively our data suggests rewiring of the TOR pathway for controlling AreA nuclear import in *A. nidulans*..

338. Role of proteolysis in the regulatory pathway of cation/alkalinity stress response in *Aspergillus nidulans*. L. Mellado, EA. Espeso. Cellular and Molecular Biology, CIB (CSIC), Madrid, Madrid, Spain.

Fungi have developed genetic strategies to survive to environment stresses, such as variations in pH, temperature, nutrient availability, reactive oxygen or diverse saline concentrations. In the filamentous fungus and model organism *Aspergillus nidulans*, tolerance to an alkaline ambient pH requires the activities of three high-hierarchy transcription factors: PacC, CrzA and SltA. We have previously described the role of SltA, a C_2H_2 zinc-finger transcription factor, in tolerance to alkalinity and to high concentrations of a number of mono and divalent cations. PacC and CrzA homologues are widely distributed among fungal kingdom but SltA homologues are found only in Pezizomycotina subphylum.

Here we present our latest results in the signaling process and the activation of SltA, in addition to its transcriptional regulatory activity. Signaling of SltA requires its proteolytic processing, an extreme post-translational modification mechanism that shares with PacC. To understand how SltA is signalized and mediates its regulatory action we have isolated mutations affecting this cation/pH response pathway. A source of new *slt*$^-$ mutations was the isolation of extragenic suppressor mutations of the lethal phenotype caused by certain null *vps* alleles. Several of these mutations mapped in *sltA* and others allowed the identification of a novel member of this pathway. The new locus has been denoted as *sltB*.

sltB gene encodes for a protein of 1272 amino acids, also specific to filamentous fungi, with two putative functional domains. The N-terminal pseudokinase domain is involved in the proteolysis of native SltA 78 kDa to a 32 kDa form. A second domain is similar to a trypsin-like protease, and our data suggest that SltB is auto-proteolyzed through this protease activity. Finally, we have determined that SltB is expressed in a SltA dependent manner. A model of regulation of SltA through SltB activity is presented for this novel cation/alkaline pH regulatory pathway in filamentous fungi.

339. Regulation and characterisation of the CreA carbon catabolite repressor in *Aspergillus nidulans*. LAURE RIES[1], GUSTAVO H. GOLDMAN[1,2]. 1) Faculty of Pharmacy, University of Sao Paulo, RIBEIRAO PRETO, Sao Paulo, Brazil; 2) National Center of Bioethanol Sciences and Technology (CTBE), CAMPINAS, Sao Paulo, Brazil.

Despite recent advances in the production of biofuels from plant biomass, readily metabolisable sugars such as glucose, released during fungal enzymatic hydrolysis of lignocellulose, still impede cellulase and hemicellulase production. In filamentous fungi, such as *Aspergillus nidulans*, glucose-mediated carbon catabolite repression (CCR) is carried out by the transcription factor CreA. Although several studies have described in detail the regulatory effects of CreA on the expression of cellulase- and hemicellulase-encoding genes, regulation of the protein itself as well as characterisation of the different CreA protein domains remains largely unknown. The aim of this study was therefore to investigate in detail the role of CreA in CCR.

Firstly, it was determined that CreA does not require *de novo* protein synthesis and is imported into the nucleus from a preformed cytoplasmic pool. Tagging CreA with the *luciferase* gene confirmed that this transcription factor is always present within the cell even in the presence of different lignocellulosic components. Deletion of four different domains of CreA resulted in strains being unable to de-repress under cellulase and hemicellulase-inducing conditions. Furthermore CreA was unable to leave the nucleus in the same conditions. One of the CreA protein regions was shown to be important for germination on cellulose and to sustain growth on complex carbon sources, ethanol and amino acids.

For the first time, CreA was shown to be always available and not completely degraded within the cell. Different domains are important for CreA nuclear localisation and one CreA protein domains appears to be important for mediating growth on a wide range of carbon and nitrogen sources.

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340. Screening for new elements involved in light regulation in *Aspergillus nidulans* by UV-mutagenesis. Z. Yu, R. Fischer. Institute for Applied Biosciences, Department of Microbiology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

Light serves as an important environmental cue for fungi, regulating development and secondary metabolism. In *Aspergillus nidulans* light is perceived by a light receptor complex consisting of the phytochrome FphA, the white-collar proteins LreA, LreB and VeA. FphA and LreA are red and blue light receptors, respectively. In light, the transcription of light-inducible genes is activated in a phytochrome-dependent manner. However, FphA does not bind to the promoters of light inducible genes directly, although a fraction of phytochrome is located in the nucleus. In order to find new elements involved in the light-response mechanism, a genetic screening approach was developed. The promoter of the light-inducible gene *ccgA* was fused to the nutritional marker gene *pyr4* and introduced into

a uracil auxotrophic strain. This strain grew like wildtype in light, but grew only very slowly in the dark. The strain was mutagenized and slow-growing colonies on medium without uracil and uridine isolated. In a second screening they were tested for the expression of light-regulated genes. From 336 slow-growing strains in light on plates without uracil and uridine, three mutants were isolated which grew like wildtype in the presence of uracil and uridine and in which *ccgA* was not induced upon light exposure. Thus they appeared to have mutations in the light-signaling cascade. The "blind" mutants could not be complemented by phytochrome. After backcrossing with wildtype, the mutations in the progenies are currently determined by multiplex genome sequencing.

341. Sensing and responding to cell wall stress in Aspergillus niger requires at least three transcription factors - RlmA, MsnA and CrzA. Markus RM Fiedler[1], Annett Lorenz[1], Benjamin M Nitsche[1], Mark Arentshorst[2,4], Cees AMJJ van den Hondel[3], Arthur FJ Ram[2,4], Vera Meyer[1,4]. 1) Applied and Molecular Microbiology, Institute of Biotechnology, Berlin, Germany; 2) Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Sylviusweg 72, 2333 BE Leiden, The Netherlands; 3) HiTeXacoat, Waterlelie 124, 2804 PZ Gouda, The Netherlands; 4) Kluyver Centre for Genomics of Industrial Fermentation, P.O. Box 5057, 2600 GA Delft, The Netherlands.

Cell wall integrity, vesicle transport and protein secretion are key factors contributing to the vitality and productivity of filamentous fungal cell factories such as Aspergillus niger. Transcriptomics signatures of A. niger and phenotypic analyses of selected null mutant strains were used to predict regulator proteins mediating the survival responses against compounds interfering with the cell wall integrity including caspofungin, aureobasidin A, fenpropimorph and FK506. This integrated approach allowed us to reconstruct a model for the cell wall salvage gene network of A. niger that ensures survival of the fungus upon cell surface stress. The model predicts that (i) caspofungin and aureobasidin A induce the cell wall integrity pathway as a main compensatory response via induction of the Rho-GTPases RhoB and RhoD, respectively, eventually activating the mitogen-activated protein kinase kinase MkkA and the transcription factor RlmA. (ii) RlmA is the main transcription factor required for the protection against calcofluor white but it cooperates with MsnA and CrzA to ensure survival of A. niger when challenged with caspofungin or aureobasidin A. (iii) Membrane stress provoked by aureobasidin A via disturbance of sphingolipid synthesis induces cell wall stress, whereas fenpropimorph-induced disturbance of ergosterol synthesis does not. The present work uncovered a sophisticated defence system of A. niger which employs at least three transcription factors - RlmA, MsnA and CrzA - to protect itself against cell wall stress. The transcriptomic data furthermore predicts a fourth transfactor, SrbA, which seems to be specifically important to survive fenpropimorph-induced cell membrane stress.

342. Possible roles of hydrophobic surface interacting proteins in the breakdown of lignocellulose by *Aspergillus niger*. R. Raulo, J. van Munster, P. Daly, R. Fetherston, M. Kokolski, D. Archer. Life Sciences, Unviersity of Nottingham, Nottingham, United Kingdom.

Enzymes from filamentous fungi have a key role in the degradation of many of the biopolymers found in nature, including cellulose and hemicelluloses. For this reason, these enzymes are of great interest in the industrial conversion of lignocellulose into biofuels. RNA-sequencing analysis has been carried out to investigate the transcriptional changes that occur when *Aspergillus niger* is transferred from the simple carbon source glucose onto the complex lignocellulosic biomass wheat straw. This has highlighted the up-regulation in transcript level of genes encoding glycosyl hydrolase (GH) enzymes as well as hydrophobic surface interacting proteins (HSIPs) that may be involved in the interface between lignocellulosic biomass and *A. niger*. Possible roles for the HSIPs include: mediating GH enzyme-substrate interactions, fungal-substrate interactions and the induction of the fungal response to lignocellulose.

To investigate the role of HSIPs in the response of *A. niger* to wheat straw, single gene deletion strains for *hfbD*, *hyp1* and *hsbA* as well as the double deletion strain (*hfbD hyp1*) have been constructed. The expression of selected genes encoding GH enzymes was then followed in these strains using qRT-PCR. The results showed that the transcript levels from the GH genes studied were lowered in the HSIPs deletion strains when compared to the wild-type strain, when the cultures were transferred from glucose medium to wheat straw. Also, a role for pH in regulating the expression of GH-encoding genes became apparent because variations in pH in cultures were observed between the different HSIPs deletion strains. As the expression of some GH-encoding genes is known to be pH-dependent, pH could be an important factor for efficient saccharification of wheat straw in *A. niger*. The precise nature of such a role is under further investigation and may provide new areas of improvement for industrial processes for production of second generation biofuels.

343. The role of carbon starvation in the induction of enzymes that degrade plant-derived carbohydrates in *Aspergillus niger*. Jolanda van Munster[1], Paul Daly[1], Stephane Delmas[1], Steven Pullan[1], Martin Blythe[2], Sunir Malla[2], Matthew Kokolski[1], Xiaolan Yu[3], Paul Dupree[3], David Archer[1]. 1) School of Life Sciences, University of Nottingham, Nottingham, United Kingdom; 2) Deep Seq, Faculty of Medicine and Health Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, United Kingdom; 3) Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.

The saccharification of lignocellulosic biomass for the production of second generation biofuels requires cheaper and more effective enzyme mixtures. Fungi are an important source of such enzymes, but the understanding of the regulation and induction of the encoding genes is still incomplete. To explore the induction mechanism, we analysed the response of the industrially important fungus *Aspergillus niger* to wheat straw, with a focus on events occurring shortly after exposure to the substrate. RNA sequencing showed that the transcriptional response after 6h of exposure to wheat straw was different from the response at 24h of exposure: the complexity increased over time.

Importantly, the influence of carbon starvation during lignocellulose degradation was demonstrated by a substantial overlap in CAZyme-encoding transcripts induced during both early carbon starvation and early exposure to straw. The up-regulation of the expression of a high number of genes encoding CAZymes that are active on plant-derived carbohydrates during early carbon starvation suggests that these enzymes could be involved in a scouting role during starvation, releasing inducing sugars from complex plant polysaccharides. We show that carbon-starved cultures indeed release CAZymes with predicted activity on plant polysaccharides. Analysis of the enzymatic activity

and the reaction products, indicates that these proteins are enzymes that can degrade various plant polysaccharides to generate both known, as well as potentially new, inducers of CAZymes (van Munster, Daly *et al,* (2014) Fungal Genet. Biol. 72; 34-47).

344. Choose On or Off: A regulatable gene expression system for filamentous fungi. Franziska Wanka, Vera Meyer. Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany.

The inducible tetracycline-dependent expression system is a versatile tool to control and fine-tune gene expression in eukaryotic cells in a metabolism-independent manner. By the addition of doxycycline, genes can either be switched on (Tet-on system) or switched off (Tet-off system). Recently, the Tet-on system has successfully been established for *Aspergillus niger* (Meyer et al., 2011) and proven to be useful for high-level production of secondary metabolites (Richter & Wanka et al., 2014).

Here, we present an optimum Tet-off system for use in *A. niger.* Both, amino acid sequence and medium-level expression of the Tet off repressor were shown to be crucial for efficient down-regulation of a gene of interest as well as for keeping the system stable in the genome of *A. niger*. We furthermore show that a gene of interest can be down-regulated in a Dox-dependent manner. This opens the possibility to establish and analyse gene-knockdown and gene-knockout mutants in *A. niger*.

Meyer et al. (2011) Appl Environ Microbiol 77:2975-83

Richter & Wanka et al. (2014). Fungal Biology and Biotechnology 1:4

345. The development of promoter shutoff system for functional analysis of essential genes with sorbitol metabolic pathway gene promoter in *Asepergillus oryzae*. S. Terado, K. Oda, A. Shimabara, R. Toyoura, M. Kawauchi, H. Fukuda, K. Iwashita. Fundamental Res. Division, Nat. Res. Inst. of Brewing, Hiroshima, Japan.

The genome sequence of several filamentous fungi revealed that almost half of genes in genome are functional unknown. So the demand of analyzing such functional unknown gene has been increased but some of the genes are essential. The promoter shutoff is general way for analyzing such essential genes. In *A. nidulans,* several conditional promoters such as *alcA* exist and showed usefulness for molecular biological studies, while in *A.oryzae* there are a few promoters which can be tightly controlled. To expand the limitation of conditional promoter use in *A.oryzae,* we explored about sorbitol and galactose metabolic pathway gene for expecting tight regulation and the less physiological effect with conditional change. We performed microarray analysis in the sorbitol or galactose (induced condition) and glucose (repressed condition) culture with *A.oryzae* RIB40 strain. Two genes are found with over 50-fold induction at sorbitol culture condition, and designated as *sorA* and *sorB* gene. To evaluate the induction with both gene promoters, we developed the EGFP expression system under the *sorA* promoter (P*sorA*) and P*sorB* control. Blight EGFP fluorescence and expression were detected at sorbitol condition, while not detected at glucose repressed condition in transformants. In addition the promoter of *brlA* which is a master regulator of the conidiation was replaced by the P*sorA* and P*sorB,* and the sorbitol dependent conidia formation was observed. This result indicated the useful of P*sorA* and P*sorB* for promoter shutoff.

To apply this promoter shutoff system for the functional analysis of essential gene, we constructed inducible PsorA::*rhoA* which is known as the essential regulator of the beta-1,3-glucan synthase. PsorA::*rhoA* strain showed complete growth inhibition at repressed condition while the strain grew normal at the induction condition, indicating this system is available for essential gene analysis. This promoter shutoff system can be further applied for essential functional unknown gene.

346. Activation mechanisms of two transcription factors, AmyR and MalR, involved in amylolytic enzyme production in *Aspergillus oryzae*. Mizuki Tanaka, Kuta Suzuki, Takanori Ichikawa, Yui Konno, Sakurako Ichinose, Sachiko Hasegawa-Shiro, Takahiro Shintani, Katsuya Gomi. Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan.

The production of amylolytic enzymes in *Aspergillus oryzae* is induced in the presence of starch or maltose, and two Zn_2Cys_6-type transcription factors, AmyR and MalR, are involved in this regulation. AmyR directly regulates the expression of amylase genes, and MalR controls the expression of maltose-utilizing (*MAL*) cluster genes encoding functional maltose permease (MalP) and maltase (MalT). To elucidate the activation mechanisms of these two transcription factors in amylase production, the expression profiles of amylases and *MAL* cluster genes under carbon catabolite derepression condition and subcellular localization of these transcription factors fused with a GFP were examined. Glucose, maltose, and isomaltose induced the expression of amylase genes, and GFP-AmyR was translocated from the cytoplasm to the nucleus after the addition of these sugars. Rapid induction of amylase gene expression and nuclear localization of GFP-AmyR by isomaltose suggested that this sugar was the strongest inducer for AmyR activation. In contrast, GFP-MalR was constitutively localized in the nucleus and the expression of *MAL* cluster genes was induced by maltose, but not by glucose or isomaltose. In the presence of maltose, the expression of amylase genes was preceded by *MAL* cluster gene expression. Furthermore, deletion of the *malR* or *malP* genes resulted in a significant decrease in the α-amylase activity induced by maltose, but had apparently no effect on the expression of amylase genes in the presence of isomaltose. These results suggested that activation of AmyR and MalR is regulated in a different manner, and the preceding activation of MalR is essential for the utilization of maltose as an inducer for AmyR activation. Investigation of the role of MalT in amylolytic genes expression is underway.

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347. The possibility of regulation system of gene expression by natural antisense transcripts in *Aspergillus oryzae*. Masaru Tsujii, Satoshi Okuda, Michio Takeuchi, Youhei Yamagata. Applied Biological Science, Tokyo University of Agriculture and Technology, fuchu, tokyo, Japan.

EST (Expressed sequence tag) analysis of *Aspergillus oryzae* have been conducted in various conditions. Analyzing of ESTs from various libraries revealed the existence of conidia specific antisense transcripts assembled to gene AO090038000160. Sequence analysis of transcripts from the gene termed *cspA* (conidia specific protein) confirmed that *cspA* sense RNA coded 444 amino acid residue protein. The protein contained catalytic domain conserved in SDRs (Short-chain dehydrogenases/reductases) and NAD binding domain. On the other hand, it was revealed that *cspA* antisense RNA could not code protein longer than 60 amino acid residues. The transcriptional region of *cspA* antisense RNA was confirmed by 5'- and 3'- RACE (rapid amplification of cDNA ends) methods. The results indicated that gene *cspA* and the antisense RNA overlapped at their 3' ends. These results suggested that gene *cspA* had NAT (natural antisense transcripts) which showed tail-to-tail format. Results of the real-time PCR demonstrated that both the sense RNA and the antisense RNA were abundant in conidia but not in germinated conidia and mycelia. Deletion of *cspA* gene, causing defect of both the sense RNA and the antisense RNA gave rise to no significant changes in phenotype. Overexpression of *cspA* sense RNA inhibited conidiation and mycelial growth, while *cspA* antisense RNA overexpression did not cause changes in phenotype. The results suggested that there might be a large amount of conidia specific NAT (natural antisense transcript) of *cspA* gene, whose product could affect conidiation and mycelial growth in *Aspergillus oryzae*. We presumed the possibility of regulation system of *cspA* expression by its NAT.

348. Brown Rot Fungus *Postia placenta* and its Differential Expression as it Degrades Wood. J.W. Zhang, G. Presley, J. Schilling. Bioproducts and Biosystems Engineering, Saint Paul, MN.

Brown rot fungi appear to have converged from diverse basidiomycete lineages to a similar carbohydrate-selective approach for degrading wood. This mechanism is of great interest for bioprocessing plant tissues industrially, but it also has clear implications on global carbon cycling. Most of these brown rot fungi lack synergistic exo-acting cellobiohydrolases as well as lignolytic class II peroxidases, unlike most white rot and cellulase-producing ascomycete fungi. Using temporally- and spatially-separated decay stages in a directional growth wood wafer design, we studied the process of wood decomposition by the model brown rot fungus *Postia placenta.* Contrary to transcription patterns for 9 Glycoside Hydrolases (GHs), 8 oxidative-treatment genes of putative or debated oxidative function (i.e. reactive oxygen species (ROS) production) were accumulated in the initial decay stages, ahead of the GH transcripts tested. Even with few cellulase transcripts, cellobiose was detected in early decay stages/locations. Further experiments showed that transcripts of mannase Man5a, xylanase Xyn10a-1 and Xyn10a-2 were significantly induced by spruce and by cellulose while repressed by monosaccharides (xylose, etc.). This contrasted carbon source effects on transcripts in the cellulolytic system, including Cel5a, Cel5b, Cel12a, Bgl1, and Bgl2, although they were not clearly repressed by glucose. In addition, putative oxidative gene transcripts such as H_2O_2-producing alcohol oxidase and copper radical oxidase were repressed by monosaccharides. Aryl-alcohol oxidase, glycopeptide and quinone reductase showed a decrease with increased mycelial age. These data support partitioning of ROS pretreatment and (hemi)cellulase saccharification genes in *Postia placenta* during wood decay process, and that temporal/spatial regulation might be influenced by the derivatives from lignocellulose decomposition such as monomeric sugars. Given the putative nature of the gene targets, however, this does not solve the brown rot mechanism; rather, it offers a ‘winnowing’ approach to begin targeting those genes of most interest in global molecular analyses.

349. Deregulation of sexual/asexual development: novel extra-circadian functions for the FREQUENCY protein in *Botrytis cinerea*? Paulo Canessa[1,2], Hanna Muller[1,2], Montserrat Hevia[1,2], Luis Larrondo[1,2]. 1) Millennium Nucleus for Fungal Integrative and Synthetic Biology (FISB); 2) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

We have recently demonstrated that genetic disruption of the *Botrytis cinerea* circadian clock by ablation of the main negative element FREQUENCY (BcFRQ1), abolishes circadian regulation of fungal virulence in this fungus. In *Neurospora crassa*, as a central component of the pacemaker, FRQ drives clock-controlled functions and does not impact other aspects of Neurospora’s biology. When analyzing growth of the *B. cinerea bcfrq1* mutant under different culture conditions, we observed an important developmental abnormality leading to the production of sexual structures such as microconidia and sclerotia. Importantly, these structures were observed under environmental conditions (light:dark cycles) that normally should lead to the production of asexual macroconidia. These phenotypes are intriguing, since they do not seem to be related to clock effects, suggesting extra-circadian functions for the BcFRQ1 protein. In order to explore these new BcFRQ1 roles on *B. cinerea* biology, we have performed global gene expression analysis of the mentioned mutant, and compared those to the wild type strain. In the absence of light, 267 and 153 genes were up and down regulated, respectively, in the *bcfrq1* mutant genetic background. Functional categorization allowed us to identify several oxidative stress related genes among up-regulated transcripts, while several metabolic-related genes were overrepresented among down-regulated transcripts. On-plate oxidative-stress resistance assays showed no significant differences between the B05.10 WT and the *bcfrq1* mutant strains. Nevertheless, complete medium breakdown allowed us to identified an important deficiency in nutrient metabolism and/or uptake that correlates with the mentioned developmental phenotype. We are now examining defects in signaling pathways, as well as developmental-specific genes in order to elucidate the mechanisms behind the mentioned phenomena. Fondecyt 1131030 and MN-FISB 120043 to LL, Fondecyt 11140678 to PC.

350. Genetic analysis of the natural resistance to the fungicide fenhexamid in the phytopathogenic fungus *Botrytis pseudocinerea:* target or not? Saad Azeddine, Alexis Billard, Jocelyne Bach, Colette Audeon, Catherine Lanen, Daniele Debieu, Sabine Fillinger. UMR BIOGER, INRA, AgroParisTech, Thiverval-Grignon, France.

The *Botrytis* species complex responsible for grey mould disease on grapevine is composed of two species: *Botrytis cinerea* the major one (about 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, one of the

most effective antibotryticides. While *B. cinerea* is sensitive to this hydroxyanilide, *B. pseudocinerea* is naturally resistant. Enzyme assays showed that in *B. pseudocinerea* the target enzyme, 3-ketoreductase involved in sterol biosynthesis was less sensitive to fenhexamid (Debieu *et al*., 2013). In addition, a strong synergism between fenhexamid and sterol 14α-demethylation inhibitors (DMIs) known to inhibit Cyp51, a cytochrome P450 monooxygenase was observed in *B. pseudocinerea*. It was thus hypothesized that detoxification of fenhexamid by a cytochrome P450 monooxygenase similar to Cyp51 could also be involved in *B. pseudocinerea's* resistance. In this work we studied separately the effect of *B. pseudocinerea's erg27* gene encoding 3-ketoreductase, and of a recently identified cytochrome P450 gene, *cyp684*, on resistance to fenhexamid.

We evaluated the contribution of fenhexamid's target protein to resistance by introducing the *B. pseudocinerea erg27* gene into a *B. cinerea* sensitive strain, replacing the natural gene. Preliminary results indicate that the gene replacement reduced *B. cinerea's* sensitivity to fenhexamid, suggesting that the target gene only plays a minor role in resistance. The *cyp684* gene showing the strongest similarity to *cyp51* among all cytochrome P450 genes was found strongly overexpressed in the presence of fenhexamid in *B. pseudocinerea.* After its deletion in a *B. pseudocinerea* strain the c*yp684* knock out mutants exhibited a strong reduction of fenhexamid resistance and synergism between DMIs and fenhexamid, indicating that the Cyp684 protein is a major player in *B. pseudocinerea's* natural resistance to fenhexamid.

351. Secondary metabolism and necrotrophy in *Botrytis cinerea*:Role of the BOA13 transcription factor. Antoine Porquier, Bérengère Dalmais, Hind Sghyer, Adeline Simon, Guillaume Morgant, Pascal Le Pecheur, Muriel Viaud. BIOGER, INRA, Avenue Lucien Brétignières 78850 Thiverval-Grignon, France.

Botrytis cinerea is the necrotrophic fungus responsible for the grey mold disease affecting more than 200 plant species. This phytopathogenic agent produces a wide range of secondary metabolites (SMs), including the phytotoxic polyketide botcinic acid (BOA). The molecular mechanisms that regulate the expression of the genes at the origin of those SMs remain unknown. The genes responsible for BOA production are organized into a cluster and one of them (*boa13*) encodes a Cys6 zinc-finger transcription factor (TF). A global transcriptomic analysis of the *Δboa13* mutant confirmed the expected control of the BOA cluster but unexpectedly, other genes more widely involved in the necrotrophic process (e.g. plant cell wall degrading enzymes) were also deregulated. As a consequence, the *Δboa13* mutant is significantly impaired in virulence. This result is fairly original as it highlights the control of genes unrelated to secondary metabolism by a TF present within a secondary metabolism cluster. The work in progress aims to (i) identify the DNA fixation patterns of BOA13 and (ii) to determine whether this TF interacts directly with the promoters of all the deregulated genes. For this purpose, a fluorescence microscopy assay of some promoters of the BOA cluster was carried out using GFP. The results suggested the implication of a candidate pattern that is present in the promoters of most of the 17 *boa* genes. A yeast-one-hybrid strategy is currently used to confirm the likely physical interaction between BOA13 and the pattern identified by the microscopy assay. The whole one-hybrid collection of *B. cinerea*'s TFs will also be screened in order to check if some of them are able to interact with the studied patterns. In parallel, the implication of a chromatin structure-based regulation of BOA genes cluster as well as few other secondary metabolism related genes is currently under study. All those results will provide a better understanding of the secondary metabolism and more widely of the necrotrophic process regulation in *B. cinerea*.

352. Adaptation to pH and pH signaling in the phytopathogenic fungus *Botrytis cinerea*. Christine RASCLE, Geneviève BILLON-GRAND, Cindy DIERYCKX, Vincent GIRARD, Nathalie POUSSEREAU. UMR5240, University Lyon 1, CNRS, Bayer SAS : 14 impasse Pierre Baizet, BP 99163, 69263 Lyon Cedex 09, France.

The infection strategy developped by *Botrytis cinerea*, an important fungal plant pathogen, is based partly on the modulation of ambient pH to ensure an optimal environment for virulence factors. *B.cinerea* is known as an « acidic « fungus since the first steps of the colonization process are associated with a pH decrease resulting from an accumulation of several organic acids. Fungi adapt to pH variations via the highly conserved signaling pathway Pal/Pac that leads to the activation of the zinc finger transcription factor PACC. Under alkaline/neutral conditions, this transcriptional regulator acts as an activator of genes expressed in alkaline conditions and a repressor of those expressed in acidic conditions.

The homologues of the seven components of the pathway were identified in the genome of *B. cinerea* and a functional analysis of *PacC* gene was performed. The deletion mutant reveals a pleiotropic phenotype and an alteration of the virulence was observed. The role of this regulator in the biology of the fungus was investigated with a focus on the different steps characteristic of the infectious cycle of *B.cinerea*.

353. Light-responsive transcription factors (LTFs) regulate differentiation and virulence in the gray mold fungus *Botrytis cinerea*. Kim Cohrs, Julia Schumacher. IBBP, WWU Münster, Schlossplatz 8, 48143 Münster, Germany.

Botrytis cinerea is the causal agent of gray mold diseases in a range of dicotyledonous plant species. The fungus reproduces asexually by forming macroconidia for dispersal and sclerotia for survival; the latter also participate in sexual reproduction by bearing the apothecia after fertilization by microconidia. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. The fungus responds to different wavelengths of light i.e. to near-UV, blue, red and far-red light suggesting that the eleven photoreceptors (two cryptochromes, four LOV proteins, two opsins, three phytochromes) are involved in light perception and initiation of downstream signaling events. Microarray analyses revealed 293 light-responsive genes including six TF-encoding (LTFs) and seven photoreceptor-encoding genes. The White Collar complex (WCC) formed by two GATA-type TFs is crucial for the response to blue/ white light as it induces the transcriptional response of many genes (Canessa et al. 2013). However, light induction of some genes still occurs in its absence and the complex also works as repressor of light-induced genes indicating the contribution of other wavelengths/ photoreceptors to drive white light-responsive gene expression. LTFs are considered regulators of differentiation in *B. cinerea* by either promoting

conidiation and/ or repressing sclerotial development. In fact, functional characterization of BcLTF1 - 3 (orthologs of *Neurospora crassa* SUB-1, SAH-1, and CSP-1) confirmed their involvement. Thus, BcLTF1 functions as repressor of conidiation and is furthermore involved in virulence, oxidative stress response and secondary metabolism (Schumacher et al. 2014). BcLTF2 appears to be the master regulator of conidiation in this system as its overexpression is sufficient to induce conidiation while its deletion abolishes conidiation. BcLTF3 is required for the differentiation of the conidia; its deletion results in malformed conidiophores that form sterile hyphae instead of conidia. As mutations of the LTFs affect the expression levels of other LTF-encoding genes, we assume a comprehensive network of TFs and photoreceptors that regulates light responses in *B. cinerea*.

354. Comparative analysis of gene regulatory networks in *Candida* species. Qinxi Ma, Matthew Dorman, Mihaela Ola, Geraldine Butler. Conway Institute, University College Dublin, Belfield, Dublin, Ireland.

The *Candida* clade (species in which CTG is translated as serine rather than leucine) contains several human fungal pathogens. There are many studies characterizing virulence of *Candida albicans*, but less is known about other members of the clade. *C. albicans* is unusual in that it can switch growth from yeast to a fully filamentous (hyphal form). *Candida parapsilosis* is the second or third most common cause of candidiasis. It exhibits polymorphic growth, but does not generate true hyphae. Here we compare the response of *C. albicans* and *C. parapsilosis* to growth on medium chain fatty acids, a source of potential antifungal drugs. We use collections of transcription factor knockouts to identify the regulators in both species. More than 20 transcription factors are required for resistance. We find that the functions of some (e.g. Bcr1, Mnl1) are conserved in both species but others (e.g. Dal81) are specific to one. We also find some changes in function between *Saccharomyces cerevisiae* and *Candida* species. RNA-seq analysis shows that exposure to fatty acids results in major metabolic changes in *Candida*, including up-regulation of fatty acid oxidation and nucleotide metabolism, and downregulation of glycolysis. Conserved mechanisms include response to weak acid stress, iron metabolism, polyamine transport and fatty acid metabolism.

355. The heat shock response governed by Hsp90 and Hsf1 is necessary for cell survival and virulence in the pathogenic fungus *Candida albicans*. Michelle Leach[1,2], Rhys Farrer[3], Koon Ho Wong[4], Christina Cuomo[3], Al Brown[2], Leah Cowen[1]. 1) Molecular Genetics Dept, University of Toronto, Toronto, Canada; 2) University of Aberdeen, IMS, Aberdeen, UK; 3) Broad Institute, Cambridge, MA, USA; 4) Faculty of Health Sciences, University of Macau, Macau.

Temperature is a ubiquitous environmental variable, adaptation to which is necessary for survival in all organisms. Upon exposure to a sub-lethal heat shock in yeast, normal metabolic functions become repressed and the heat shock transcription factor Hsf1 is activated, inducing heat shock proteins (HSPs). *Candida albicans*, the most prevalent human fungal pathogen, is an opportunistic pathogen that has evolved as a harmless commensal of healthy individuals. Even though *C. albicans* occupies thermally buffered niches, it has retained the classic heat shock response, activating Hsf1 during slow thermal transitions such as increases in temperature suffered by febrile patients. The molecular chaperone Hsp90 interacts with and down-regulates Hsf1 in *C. albicans*, modulating short-term activation of the heat shock response. To obtain a global picture of the heat shock response we have performed both RNA-seq and ChIP-seq in the absence and presence of heat shock to determine which genes Hsf1 binds and regulates. As expected, Hsf1 binds to and regulates heat shock proteins necessary for cell survival upon a heat shock. However, a subset of genes required for virulence was also upregulated and bound by Hsf1. These genes are not required for survival at high temperatures, with mutants lacking these genes displaying no growth defect upon heat shock. Our data suggest that Hsf1 is not only essential for regulating genes necessary for cell survival upon heat shock, but also genes required for virulence. Indeed, cells that have received a sub-lethal heat shock are more virulent in a *Galleria mellonella* model of infection compared to cells grown at 30°C. Finally, we show that depletion of *HSP90* drastically changes the signature of the heat shock response, blocking upregulation of many of these virulence genes. Therefore, Hsf1 and Hsp90 act in concert in the pathogen to combat the host response of fever by upregulating genes necessary for cell survival and continued infection.

356. Elucidating the mechanism through which Cas5 regulates caspofungin tolerance in *Candida albicans*. Jinglin L. Xie[1], Michelle D. Leach[1,2], Jonathan R. Krieger[3], Jiefei Tong[4], Michael F. Moran[1,4], Leah E. Cowen[1]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Aberdeen Fungal Group, School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Abderdeen, United Kingdom; 3) The Hospital for Sick Children, SPARC Biocentre, Mass Spectrometry Facility, Toronto, Ontario, Canada; 4) The Hospital For Sick Children, Program in Molecular Structure and Function, Peter Gilgan Centre for Research and Learning, Toronto, Ontario, Canada.

Candida albicans is a leading causative agent of fungal infections in humans. Resistance to current antifungal drugs arises frequently and is a major clinical problem. Development of new antifungal drugs remains a difficult process, partly due to the conservation of many potential therapeutic targets between *C. albicans* and humans. Moreover, stress responses in *C. albicans* enhance antifungal tolerance and enable drug resistance. Therefore, tactical targeting of specific stress response pathways in combination with antifungal agents may provide a viable strategy to suppress the emergence of antifungal drug resistance. In a recent study, the transcription factor Cas5 was identified in a screen for genes required for tolerance to the echinocandin caspofungin, a clinically prescribed fungal cell wall synthesis inhibitor. The goal of my project is to uncover the dynamic regulation of Cas5 in response to caspofungin-induced cell wall stress. We have confirmed that Cas5 is required for the induction of cell wall-related gene expression in response to caspofungin by qRT-PCR. Western blot analysis shows that Cas5 is post-translationally modified in response to caspofungin and that its activity is modulated by cell wall state. Immunoprecipitation coupled with mass spectrometry identified a number of Cas5 interactors, including the SBF complex members Swi4 and Swi6. We are currently in the process of identifying the post-translational modification on Cas5 and mapping the Cas5-dependent transcriptional outputs required for response to cell wall stress. Our work illuminates a novel pathway involved in regulating antifungal drug tolerance and suggests potential new avenues for combination therapies with echinocandin antifungal agents.

357. Epigenetic hotspots are genomic islands for putative effector-encoding genes in *Zymoseptoria tritici*. Jessica L. Soyer[1,2,3], Jonathan Grandaubert[2,3], Klaas Schotanus[2,3], Janine Haueisen[2,3], Eva H. Stukenbrock[2,3]. 1) INRA UR 1290 BIOGER - CPP Avenue Lucien Brétignières, BP 01, 78850 Thiverval-Grignon, France; 2) Max-Planck-Institut for Evolutionary biology, August-Thienemann-Str. 2, 24306 Plön, Germany; 3) Christian-Albrechts University of Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany.

The Dothideomycete *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*) is a pathogen of wheat (*Triticum aestivum*) and has a hemibiotrophic life style suggesting a fine-tuned regulation of gene expression. The genome of *Z. tritici* comprises 21 chromosomes including up to eight conditionally dispensable chromosomes (CDCs) containing a considerably higher proportion of transposable elements (TEs) than core chromosomes (Goodwin *et al*., 2011). Evidence is accumulating that genes involved in host-pathogen interaction, such as effector-genes, can be located in TE-rich, heterochromatic genomic regions. In *Z. tritici*, while CDCs are TE-rich, they harbor few effector-genes. We however hypothesized that effector-genes are not randomly located on core chromosomes. In order to investigate their location with respect to the chromatin structure, a genome-wide histone map was established using ChIP-sequencing and correlated with TE, effector-gene location and transcriptomic data. As expected, CDCs are mainly heterochromatic while gene-rich core chromosomes are mainly euchromatic, which is consistent with the low expression of genes located on CDCs compared to core chromosomes. TE-rich regions of the core chromosomes are also enriched in histone modifications typical of heterochromatic domains, suggesting that an epigenetic control of expression of the genes located in these regions could occur. We define these regions of the core chromosomes as "epigenetic hotspots". Analysis of the location of effector-genes in relation to epigenetic hotspots highlighted that these genes are significantly associated with heterochromatic domains. We further investigated effect of this location on the regulation of expression of these pathogenicity-related encoding genes during two stages of wheat infection. We conclude that TE-rich regions of core chromosomes define genomic environments for putative effector-encoding genes and that histone modifications may control their expression.

358. Interconnected network of circadian rhythms and DNA damage response. Judit Zamborszky, Toru Matsu-ura, Jaesang Kwon, Mokryun Baek, Christian Hong. Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH.

The maintenance of genome integrity is essential for organisms. Recent findings indicate bidirectional influence between DNA damage response (DDR) and circadian rhythms. In both *Neurospora crassa* and *Mus musculus*, activated DNA damage response checkpoint kinase, PRD-4 (CHK2), phosphorylates a core clock components (i.e. FRQ in Neurospora and PER1 in mouse) and induces phase advances of circadian rhythms. On the other hand, circadian rhythms modulate ATR-mediated DNA damage response. However, detailed understanding of this network between circadian rhythms and DNA damage response remain elusive. In this report, we demonstrate circadian gene expression of key DDR components, *mus-21* (ATM) and *prd-4* (CHK2) in *Neurospora crassa*. These oscillations are abolished in circadian arrhythmic mutant, frq^{ko}. More importantly, rhythmic expression of *mus-21* and *prd-4* result in distinct circadian time-dependent DNA damage responses, which transiently disrupts conidiation-banding patterns upon DNA damage. Our findings unravel optimized circadian clock-dependent operations of DNA damage response mechanisms via *mus-21* and *prd-4*.

359. Two circadian oscillators function to coordinately regulate circadian rhythmicity in *Neurospora crassa*. Nirmala Karunarathna[1], Renato de Paula[1], Bin Wang[2], Jay Dunlap[2], Deborah Bell-Pedersen[2]. 1) 3258 TAMU, Biological Sciences Building West, College Station, TX; 2) Dartmouth Medical School, Hanover, NH.

Most eukaryotes possess a circadian timing mechanism composed of at least a core circadian oscillator that uses proteins with opposing functions to form a negative feedback loop: positive-acting elements stimulate expression of the negative-acting elements, and the negative elements physically interact with the positive elements to inhibit their function. While the molecular details of the core oscillator are well described, new data suggests that the clock is comprised of multiple oscillators. For example, rhythmic behavior has been observed in canonical clock mutants of Neurospora, Drosophila, and mammals. In Neurospora, oscillations that are unmasked when the core circadian oscillator, the FRQ/WC oscillator (FWO), is eliminated have been referred to as FRQ-less oscillations that are driven by FRQ-less oscillators (FLOs).We recently discovered a set of genes, including *ccg-16* of unknown function, which are rhythmic in constant dark in strains that lack FRQ, and thus a functional FWO, but which require the WCC for rhythmicity. In addition, mRNA and protein levels of *ccg-16* cycle in constant light, conditions in which the FWO is arrhythmic. These data indicated the existence of a second oscillator in Neurospora cells, called the WCC-FRQ-less oscillator (WC-FLO), which may be coupled to the FWO through the WCC. We hypothesized that components of the WC-FLO physically interact with the WCC, similar to the interaction of FRQ with the WCC required negative feedback of the FWO. To test this hypothesis, knockouts of genes encoding WCC interacting proteins were examined for loss of *ccg-16:luciferase* reporter rhythms. Deletion of a WCC-interacting and clock-controlled transcription factor abolished *ccg-16* rhythms, suggesting a role for this protein in the WC-FLO mechanism, or in an output pathway from the WCC-FLO. Experiments are in progress to distinguish between these possibilities.

360. Analysis of circadian rhythms in the basal filamentous ascomycete *Pyronema confluens*. Stefanie Traeger, Minou Nowrousian. Dept. of General & Molecular Botany, Ruhr University Bochum, Bochum, Germany.

Many organisms use a circadian clock system to adapt to daily changes in the environment. Major insights into the molecular mechanisms of circadian oscillators have been gained through studies of the model organism *Neurspora crassa*; however, little is known about circadian clocks in other fungal species. An important component of the Neurospora circadian oscillator is the *frq* (*frequency*) gene, homologs of which can be found in the genomes of Sordariomycetes, Dothideomycetes, and Leotimycetes, but not Eurotiomycetes. Recently, we sequenced the genome of *Pyronema confluens*, and identified a *frq* homolog in this species, the first in the early-diverging Pezizomycete lineage of filamentous ascomycetes (Traeger et al. 2013, PLoS Genet 9: e1003820). Similar to *N. crassa*, the *P. confluens frq* is transcriptionally induced by light. The finding of a *frq* homolog in a basal filamentous ascomycete opens the possibility that this species has a circadian clock, and that *frq* might be part of a putative oscillator. However, there is no morphological phenotype that shows overt circadian rhythmicity, as fruiting body development is not rhythmic, and conidiospores are lacking in *P. confluens*. To investigate whether a molecular clock is present nevertheless, we first analyzed *frq* transcription in constant darkness, and found circadian oscillation of *frq*

with a peak in the subjective morning. This rhythm was present at 25 and 30 °C (temperature compensation). To identify additional clock-controlled genes (ccgs), we performed RNA-seq of two time points (subjective morning and subjective evening). Several ccg candidates were checked by RT-qPCR over a full time course, and we were able to verify circadian expression of four morning-specific genes at 25 and 30 °C, whereas expression of five putative evening-specific genes could not be verified as circadian. Thus far, we have tested two out of three properties of a circadian clock (free-running rhythm and temperature compensation), and have shown that at least five *P. confluens* genes, including the *frq* homolog, are expressed in a manner consistent with circadian regulation.

361. Cryptococcal titan cells are generated through an atypical cell cycle during pulmonary infection. Zhongming Li, Kirsten Nielsen. Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota.

Cryptococcus neoformans is a common life-threatening fungus that causes more than 650,000 deaths annually. Upon inhalation, C. neoformans establishes an initial pulmonary infection that eventually disseminates to the central nervous system to cause meningitis. During pulmonary infection, C. neoformans produces polyploid titan cells that are critical for the establishment of pulmonary infection and subsequent dissemination. Despite the importance of titan cell production in virulence of C. neoformans, the molecular mechanisms underlying the polyploidy observed in titan cells remains unknown. Polyploid cells are very common in nature and have been found in plants, animals, anthropods, fungi etc. Polyploid cells are usually formed through an endocycle or endoreplication that uncouples DNA replication and cell division by downregulation of mitotic cyclins and cyclin-dependent kinase activity. We identified 14 putative cyclins and 7 putative cyclin-dependent kinases (CDKs) in C. neoformans based on homology to known cyclins and CDKs in Saccharomyces cerevisiae. We were able to delete twelve putative cyclins and three putative CDKs, with the rest likely essential for C. neoformans. Using an in vivo mouse model of titan cell formation, we showed a significant increase in titan cell production for three cyclin and two CDK mutants. In addition, one cyclin mutant shows a severe growth defect in vivo due to increased phagocytosis by host immune cells. These observations support a model in which cryptococcal titan cells are produced through an endocycle during pulmonary infection.

362. Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. Jessica Soyer[1], Mennat El Ghalid[1], Jonathan Grandaubert[1], Marie-Hélène Balesdent[1], Lanelle Connolly[2], Michael Freitag[2], Thierry Rouxel[1], Isabelle Fudal[1]. 1) INRA, UR1290 BIOGER, Grignon, France; 2) Biochemistry and Biophysics Dept, Oregon State University, Corvallis, USA.

Plant pathogens 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 during plant infection. To date, little is known about the regulation of their expression. *Leptosphaeria maculans* is an ascomycete fungus responsible for stem canker of oilseed rape. Its genome has a bipartite structure alternating gene rich GC-equilibrated 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 one histone modification, histone H3 lysine 9 methylation (H3K9me3), in epigenetic regulation of concerted effector gene expression in *L. maculans*. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, HP1 and DIM5, by RNAi. By using HP1-GFP as a heterochromatin marker, we observed that almost no chromatin condensation is visible in a silenced-*dim5* background. Whole genome oligoarrays performed on silenced-*hp1* and silenced-*dim5* transformants background revealed an over-expression of pathogenicity-related genes during *in vitro* growth, with a favored influence on SSP-encoding genes in AT-isochores. That increase of expression during *in vitro* growth was associated with a reduction of H3K9 trimethylation at two SSP-encoding gene loci. These data strongly suggest that an epigenetic control, mediated by HP1 and DIM5, represses the expression of at least part of the effector genes located in AT-isochores during growth in axenic culture. Our hypothesis is that changes of lifestyle and a switch toward pathogenesis lift chromatin-mediated repression, allowing a rapid response to new environmental conditions.

363. Functional characterization of class II hydrophobins, Hyd4 and Hyd6, in mycotoxigenic fungus *Fusarium verticillioides*. Angelyn Hilton, Won-Bo Shim. Plant Pathology and Microbiology, Texas A&M University, College Station, TX.

Hydrophobins are small cysteine-rich proteins specific to filamentous fungi and are known to play a number of different roles in fungal physiology. For instance, in *Magnaporthe oryzae* hydrophobins are associated with aerial hyphae formation, surface hydrophobicity, sexual and asexual reproduction, and appressorium development. Our lab is interested in *Fusarium verticillioides*, the causal agent of stalk and kernel rot in maize, which significantly produces fumonisins, a group of secondary metabolites associated with esophageal cancer, equine leukoencephalomalacia and porcine pulmonary edema syndrome, on infested kernels. The role of hydrophobins in *F. verticillioides* development, reproduction, secondary metabolism and pathogenicity is still ambiguous. Hydrophobins can be divided into two classes based on hydropathy plots, solubility and secondary structure. In *F. verticillioides*, the class I hydrophobins, Hyd1 and Hyd2, were found to be involved in microconidial chain development but not in virulence. Recently we identified two genes encoding putative class II hydrophobins in *F. verticillioides*, and the aim of this study is to characterize the functional role of these hydrophobins, Hyd4 and Hyd6. *In silico* analysis revealed that Hyd4 encodes for a 702 aa protein with a class II hydrophobin domain and a probable pectin esterase inhibitor. Meanwhile, Hyd6 encodes for a 100 aa protein with only a putative class II hydrophobin domain present. The two hydrophobins share a 64% protein homology. In addition to *in silico* analysis, we are using quantitative PCR to determine the expression pattern of Hyd4 and Hyd6 in a variety of culture conditions. Using a split marker approach, *HYD4* and *HYD6* knockout mutants were generated and verified with PCR and Southern hybridization. The knockout mutants showed little to no difference in phenotype in comparison to the wild type. Due to the high homology amongst Hyd4 and Hyd6, we hypothesize that one hydrophobin is compensating for the other in these single-knockout mutants. A *HYD4*/*HYD6* double mutant will allow us to test this hypothesis and further investigate the role of class II hydrophobins in *F. verticillioides*.

364. Regulation of Trichothecene and Fumonisin B1 Biosynthesis by $Zn(II)_2Cys_6$ Binuclear Transcription Factor. Hun Kim[1], Hokyoung Son[2], Yin-Won Lee[2]. 1) Research Center for Eco-friendly New Materials, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea; 2) Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea.

Molecular mechanisms responding to environmental factors, such as nitrogen, carbon, and pH, are connected to components regulating mycotoxin production. A few studies have reported the effects of several genes, which are involved in carbon metabolism, on fumonisin production of *Fusarium verticillioides*. However, relatively little is known about how trichothecene biosynthesis of *F. graminearum* is regulated by carbon metabolism. Previously, we generated 657 individual mutants, which genes encoding putative transcription factors (TF) are deleted. Of these mutants, in this study, we investigated fungal growth of 55 TF mutants, which showed the defects of trichothecene production, in starch liquid media, and consequently found a deletion mutant (FGSG_02083) showing growth impairment in the media. The FGSG_02083, designated as *Atr1*, is predicted to encode a $Zn(II)_2Cys_6$ zinc finger transcription factor, and nuclear localization of ATR1 protein supports its role as a transcriptional regulator. The *Atr1* deletion mutant exhibited impairment of starch hydrolysis, resulted from low expression of alpha-amylase and glucoamylase gene. Moreover, the mutant showed the defect of germination in starch liquid media, but not on the media when either glucose or sucrose was supplied instead of starch. When expression of *Tri5* and *Tri6* was measured, the genes were slightly expressed in the deletion strain, corresponding to the lower trichothecene production, whereas the complemented strain recovered the ability of trichothecene biosynthesis as the wild-type strain. To investigate roles of *Atr1* for fumonisin biosynthesis, we deleted a homolog of FGSG_02083 in *F. verticillioides*. A resulting strain, like the *Atr1* deletion mutant, showed the defects of starch hydrolysis and produced less amounts of fumonisin B1. Taken together, these results demonstrate that ATR1 TF controls both trichothecene and fumonisin biosynthesis by regulating genes involved in starch saccharification.

365. The dynamic interplay of the global nitrogen-regulators AreA and AreB on genome-wide gene expression in *Fusarium fujikuroi*. Andreas Pfannmüller[1], Lena Studt[1], Christian Sieber[2], Johannes Barth[1], Martin Münsterkötter[2], Caroline Michielse[1], Christian Fufezan[1], Michael Hippler[1], Ulrich Güldener[2], Bettina Tudzynski[1]. 1) Institute of Biology and Biotechnology of Plants, Westfälische Wilhelms-University, Münster, Germany; 2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Munich, Germany.

The global GATA transcription factors AreA and AreB play key roles in the nitrogen-dependent regulation of secondary metabolism in the notorious rice pathogen *Fusarium fujikuroi*. For example, both regulators are essential to activate expression of gibberellin and fumonisin biosynthetic genes. Recently, we have shown that both transcription factors can act as positive and negative regulators of common as well as distinct sets of target genes. Furthermore, AreA activates the transcription of *AREB*, but not vice versa. Both regulators interact in the nucleus, indicating a complex regulatory interplay between AreA and AreB. To get a deeper insight into the role of both transcription factors, we performed genome-wide transcriptome and proteome analyses of the wild type and Δ*AREA* and Δ*AREB* mutants. *In vitro* microarray analysis revealed that each transcription factor regulates a total set of about 4200 genes under nitrogen limiting conditions with an enrichment of genes belonging to functional classes of secondary metabolism, transporters, transcription factors as well as putative virulence factors. Under nitrogen-sufficient conditions, AreA is not active as a transcriptional regulator, while AreB affects expression of about 4400 genes. Both transcription factors regulate a common set of about 2100 genes. For 250 of these target genes, AreA and AreB counteract each other, but most of the times they work together either as positive or as negative regulators. Furthermore, we studied the effect of AreA and AreB deletion with regard to *in planta* gene expression (infected rice plants) by RNA-seq as well as on the proteome and phosphoproteome by coupled HPLC-MS analysis. In addition, we compared the epigenetic landscape at AreA and/or AreB-dependent SM gene clusters by chromatine immunoprecipitation (ChIP). Taken together, we provide new insights into the complex roles both GATA-factors play on the different levels of gene regulation including histone modifications.

366. SAGA/Ada complex affects primary and secondary metabolism in *Fusarium fujikuroi*. S.M. Roesler[1,2], S. Breen[3], H.U. Humpf[2], B. Tudzynski[1]. 1) Institute for Plant Biology and Biotechnology, WWU,Münster, 48149 Germany; 2) Institute of Food Chemistry, WWU, 48149 Münster, Germany; 3) Plant Science Division, Research School of Biology, ANU, Canberra 0200, Australia.

The present work focuses on the histone acetyltransferase Gcn5 as part of the SAGA/Ada complex in *Fusarium fujikuroi*. This complex is involved in transcriptional regulation of response to environmental stresses such as metabolic starvation and DNA damage in *Saccharomyces cerevisiae*. In *Aspergillus nidulans* the Gcn5 homolog GcnE and the adapter protein AdaB are essential for *Streptomyces rapamycinicus*-induced activation of the orsellinic acid gene cluster *via* H3K9 and H3K14 acetylation. In *F. fujikuroi* Δ*gcn5* and Δ*ada2* single deletion mutants show severe growth defects on solid media and in liquid culture. Production of several secondary metabolites (SMs) is altered under low or high glutamine (N) conditions. Biosynthesis of gibberellins (low N) and fusaric acid (high N) is inhibited in the deletion mutants whereas bikaverin (low N) and fusarin C (high N) production is elevated. Microarray data confirm HPLC analyses of these SMs for Δ*gcn5* at gene expression level. The data also showed deregulated expression of several transporters and transcription factors, which could explain inhibited growth and changes in metabolism. Moreover, key genes of two cryptic and silent SM clusters encoding a polyketide synthase and a sesquiterpene cyclase, respectively, were highly expressed in Δ*gcn5*. As transcriptomic analysis of *F. fujikuroi* showed many putative SM clusters are silent under standard conditions, deletion of *gcn5* is a proper tool to find new metabolites. In contrast to deletions neither single nor double overexpression of *gcn5* and *ada2* led to altered SM spectra suggesting other complex members to be essential for full functionality. To gain insight into the protein interactions in the SAGA/Ada complex of *F. fujikuroi*, a yeast 2-hybrid assay was conducted. Gcn5 showed weak interaction with Ada2 and Ada3, a further adapter protein identified in *S. cerevisiae.* Ada2 and Ada3 strongly interacted with each other, but also formed homodimers, supporting their role as scaffold proteins. This work demonstrates that Gcn5 and Ada2 are important regulators of SM, growth and differentiation in *F. fujikuroi*.

367. Identification of genes *defective in silencing* by a forward genetics approach in *Fusarium graminearum*. Lanelle R. Connolly, Xiao Lan Chang, Brett Pierce, Corinne Fargo, Kristina M. Smith, Michael Freitag. Biochem/Biophysics, Oregon State Univ, Corvallis, OR.

Polycomb group proteins induce transcriptionally silent chromatin by generating and binding trimethylated histone H3 lysine 27 (H3K27me3). The pathway was originally discovered in Drosophila and has been extensively studied in mammals. The exact mechanisms

for gene silencing remain controversial. Virtually nothing is known about this silencing pathway in the fungi. In our efforts to characterize Polycomb Repressive complex 2 (PRC2) composition and discover novel components in fungi, we identified three core components of PRC2 in *Fusarium graminearum*. We found that ~30% of the genome is silenced by H3K27me3, and that loss of this mark in three PRC2 mutants (*kmt6, suz12, eed*) results in novel or increased expression of ~20% of all genes. Most of these silenced genes are predicted to be involved in development, production of known and unknown secondary metabolites of potential importance in industry and medicine, or putative pathogenicity factors. To uncover suppressors of H3K27me3 silencing, we have developed a forward genetics approach utilizing UV mutagenesis. We constructed a neomycin-resistant reporter gene (*neo*) and inserted it in a region that is silenced by H3K27me3 in WT, but de-repressed in our PRC2 mutants. WT strains with *neo* at the heterochromatic locus were UV-irradiated, and mutants were selected on G418-containing medium. Selected mutants were analyzed by qRT-PCR and RNA-seq. Four mutants have altered patterns of gene expression, and one of these mutants shows global expression patterns almost identical to our three known PRC2 mutants. We predict that the mutation resides in a novel gene involved in H3K27me3-mediated silencing.

368. Phenotypic screens and transcriptional profiling used to identify conidiation-related genes controlling asexual development in *Fusarium graminearum*. Z. Bilton[1], H. Osunga Buyu[1], B. Sevek[1], C. Rivera[1], G. Ellison[1], R. Hirsch[1], B. Bluhm[2], J. Flaherty[1]. 1) Science and Mathematics, Coker College, Hartsville, SC; 2) Plant Pathology, University of Arkansas, Fayetteville, AR.

Our group seeks to isolate and characterize genes involved in asexual development (conidiation) using the model fungal pathogen *Fusarium graminearum*. Towards this goal, we applied phenotypic screens to identify developmentally impaired mutants of *F. graminearum* that exhibit atypical responses to environmental cues (*e.g.*, light and osmotic stress) during conidiation. Two REMI insertional mutants (designated 8E8 and 11B1), each displaying a spectrum of phenotypes, including abnormal development of conidia, were identified and characterized at the genomic level. Re-sequencing both mutant genomes (each at ~44X coverage) revealed the genetic lesions caused by integration of the REMI vector; two separate insertion events were found in 8E8 (FGSG_02455 and FGSG_10678) and a single insertion in 11B1 (FGSG_08426). Multiple independent targeted gene knockouts corresponding to the mutant loci were produced by a split-marker PCR technique. Only disruption of FGSG_02455 (not FGSG_10678) contributed to phenotypes displayed by 8E8 (aberrant conidiation dynamics, reduced colonial growth, increased pigmentation), while the FGSG_10678 mutant resembles the parent strain (PH-1). Targeted disruption of FGSG_08426 resulted in strains that display an overly osmotic sensitive phenotype and lower levels of conidiation, both phenotypes shared with the corresponding REMI mutant, 11B1. Furthermore, transcription profiling experiments using RNAseq methods (Ion Torrent, ~2 million reads per sample) were performed to compare 11B1 and PH-1 cultured in liquid YEG medium (not conducive for conidiation) and YEG+ 7% NaCl (conducive for conidiation). A genome-based RNAseq analysis using the Tuxedo Package revealed >2,400 differentially expressed genes (enriched or repressed; $P<0.001$) in PH-1 across the two culture conditions. Bioinformatic analysis of selected genes will be presented in addition to comparisons of gene expression shown for the 11B1 and PH-1 strains.

369. The Polycomb Repressive Complex 2 and facultative heterochromatin of *Fusarium graminearum*. Steven Friedman, Lanelle Connolly, Phuong Pham, Jonathan Galazka, Kristina Smith, Michael Freitag. Biochemistry & Biophysics, Oregon State University, Corvallis, OR.

The cereal pathogen *Fusarium graminearum* produces secondary metabolites toxic to humans and animals, yet coordinated transcriptional regulation of secondary metabolite (SM) gene clusters remains largely a mystery. By ChIP-sequencing (ChIP-seq) we found that regions of the *F. graminearum* genome with SM clusters are enriched for a histone modification - trimethylated histone H3 lysine 27 (H3K27me3) - that is associated with gene silencing. H3K27me3 was found predominantly in regions that lack synteny with other *Fusarium* species, generally subtelomeric regions. Di- or trimethylated H3K4 (H3K4me2/3), two modifications associated with gene activity, and H3K27me3 are predominantly 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 do deletions of two additional genes that are conserved subunits of the Polycomb Repressive Complex 2 (PRC2), *eed* and *suz12*. All three mutants display growth defects, are self-sterile, and constitutively express. genes for several mycotoxins. We tagged both KMT6 and EED with GFP and found the proteins localized in domains on the inner side of the nuclear membrane, a localization that was absent when we examined EED-GFP in a *kmt6* mutant. To further elucidate the role of KMT6 and H3K27me3 in gene silencing, we employed chromatin conformation capture followed by high-throughput sequencing (HiC) in WT and *kmt6* strains.

370. Modular and compartmentalized gene regulatory networks of *Fusarium graminearum*. Li Guo[1], Guoyi Zhao[2], Lixin Gao[2], Jin-Rong Xu[3], H. Corby Kistler[4], Li-Jun Ma[1]. 1) Department of Biochemistry and Molecular Biology, 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.

Fusarium head blight caused by filamentous ascomycete *Fusarium graminearum* (*Fg*) is a major limiting factor for global wheat production, leading to reduced yield and toxic grains containing trichothecene mycotoxins. Knowledge on the gene regulatory network (GRN) of this fungus is currently lacking but vital for understanding the pathobiology and developing effective disease management strategies. We have reconstructed an *Fg* GRN based on a collection of microarray data spanning a wide range of biological states using a Bayesian networks algorithm. The biological relevance of this *Fg* GRN is validated using prior biological knowledge of *Fg*. Our *Fg* GRN also partially overlaps with *Fg* Protein-Protein Interaction (FPPI) database, mainly covering protein complexes including a ribosomal complex and a spliceosome complex. The *Fg* GRN can be divided into eight distinct but interconnected modules that are enriched for different biological functions, such as cell cycle, protein synthesis and self-defense. These eight modules are differentially induced under different biological processes, such as pathogenesis, sexual development and conidia germination. Interestingly, we discover a distinct regulatory pattern for regulators from conserved and nonconserved genomic regions, suggesting that regulators may predominantly regulate target genes from the same genomic regions. Two regulatory modules have an overrepresentation of genes in nonconserved genomic

regions. Such patterns indicates that *Fg* genome may have evolved with diverged regulatory circuits for the two genomic regions so that regulators and target genes from the same region combine to control specialized biological functions. This first ever reconstructed *Fg* GRN not only provides insight into the cell circuits of this pathogenic ascomycete, but also lays a foundation for future experimental studies on gene regulation controlling fundamental biological functions.

371. Transcriptomics of oxidative stress response mediated by *Fgap1*. Mathilde Montibus, Enric Zehraoui, Florence Richard-Forget, Christian Barreau, Nadia Ponts. INRA, UR1264 MycSA, 71 avenue Edouard Bourlaux, CS20032, F-33882 Villenave d'Ornon Cedex, France.

The filamentous fungus *Fusarium graminearum* can infect many cereals. It is one of the main causal agents of "*Fusarium* Head Blight". 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 is not fully understood yet. 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. We previously found that *Fgap1*, encoding a protein homologue to the yeast redox-response positive transcription factor Yap1, is involved in oxidative stress response in *F. graminearum*, including the production of trichothecenes. Here, we investigate the genes involved in the response pathway mediated by *Fgap1* in *F. graminearum*. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in *F. graminearum* were used in transcriptomics studies carried on *F. graminearum* grown in GYEP liquid medium supplemented, or not supplemented, with H_2O_2. We found that Fgap1 mediates response to oxidative stress by H_2O_2. Nonetheless, an alternative pathway also exists. Indeed, in the absence of *Fgap1*, a different set of genes is activated upon stress. This gene network re-wiring highlights the intergenic connectivity dynamics that take place upon environmental stimuli.

372. Genome-wide analysis of *Fusarium graminearum*'s nucleosome landscape. Enric Zehraoui[1], Slavica Djuric-Ciganovic[2], Christof Rampitsch[2], Nadia Ponts[1]. 1) INRA, UR1264 MycSA, 71 avenue Edouard Bourlaux, CS20032, F-33882 Villenave d'Ornon Cedex, France; 2) Agriculture and Agrifood Canada, Cereal Research Centre, Morden MB, Canada.

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 enzymes that catalyze the biosynthesis of type B trichothecenes are encoded by *Tri* genes found clustered together in the genome. This so-called Tri Cluster is under the control of various external stimuli such as the light. Evidences point out chromatin structure - *e.g.*, nucleosome patterns, the deposition of histone marks – as an upper level of regulation of secondary metabolite genes, including *Tri* genes. For example, genomic regions containing secondary metabolite genes are enriched in histone H3 trimethylated on its lysine 27, typically a silencing mark (Connolly et al. 2013).

The present study analyzes *F. graminearum* chromatin structure changes during asexual development and DON production. Here, we explore the nucleosome landscape as well as the repertoire of histone post-translational modifications in *F. graminearum*. The positions of nucleosomes genome-wide was investigated by MNase-assisted isolation of nucleosomal elements coupled to deep sequencing. Nucleosomes arrays appear well set in the genome, most positions being conserved during development. The *Tri* cluster was found to contain unevenly spaced nucleosomes. Histone modifications were investigated by high-resolution mass spectrometry post derivatization. The method permitted up to 100% of sequence coverage and identified various modifications, conserved or not, including acetylations, phosphorylations, methylations, *etc*. Most modifications were found at the N-terminal end, although other residues were eventually found as modified. All together, these results propose a first draft of *F. graminearum*'s chromatin landscape at the genome scale.

373. Effector gene expression in *F.oxysporum* is regulated by the transcription factor FTF1. C. van der Does[1], A. Yang[2], L. Fokkens[1], S.M. Schmidt[1], E-J. Eggers[1], J. Lukasiewicz[1], T. Hughes[2], M. Rep[1]. 1) Molecular plant pathology, University of Amsterdam, Netherlands; 2) Banting and Best Department of Medical Research, University of Toronto, Canada.

In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on an accessory chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is massively upregulated upon infection and requires SGE1, a transcription factor encoded on the core genome. The accessory chromosome itself also contains 10 predicted transcription factor genes. Among these are three homologs of *FTF1*, and one homolog of *EBR2*. Of all transcription factor genes on the accessory chromosome, except one, there is a homolog in the core genome. We started an investigation of the possible function of the 'accessory' transcription factor genes.

To see if homologs on core and accessory chromosomes could have differentiated functions, we determined the DNA binding site (DBS) using oligonucleotide arrays. This was successful for four of the transcription factors on the accessory chromosome and three of the homologs on the core genome. The binding sites for homologs were in all cases highly similar or identical. Remarkably, the DBS for FTF1 corresponds to a motif found earlier to be enriched in the promoters of effector genes. The FTF1 DBS is significantly enriched on the accessory chromosomes, and in the promoters of genes upregulated during infection. Upon overexpression, a *FTF1* homolog from the accessory chromosome and the *FTF1* homolog on the core strongly induced expression of some effector genes. Also *SGE1*, when overexpressed, can activate effector genes, although not to the same extent as *FTF1*. The other TFs seem to have little or no effect.

We propose that FTF1 regulates effector gene expression by direct binding to the promoter. Furthermore, the core and accessory genome seem to be transcriptionally interconnected. We are in the process of performing RNAseq experiments with TF overexpression strains to unravel this transcriptional network.

374. The M35 neutral metallopeptidase NMP1 of *Trichoderma guizhouense* is required for predation on *Fusarium oxysporum* and is involved in biotrophic or antagonistic interactions with some other fungi. Jian Zhang[1,2], Gunseli Bayram Akcapinar[3,4], Lea Atanasova[3,4], Mohammad Javad Rahimi[3], Agnes Przylucka[3], Dongqing Yang[1,2], Christian P. Kubicek[3], Ruifu Zhang[1,2], Qirong Shen[1,2], Irina S. Druzhinina[3,4]. 1) plants nutrition, Nanjing Agricultural University, Nanjing, Jiangsu, China; 2) National Engineering Center for Solid Organic Waste Utilization, Yixing, China; 3) Microbiology Group, Institute of Chemical Engineering, Vienna University of Technology , Vienna, Austria; 4) Austrian Centre of Industrial Biotechnology (ACIB) GmbH, Graz, Austria.

Trichoderma guizhouense, a sister species to *T. harzianum*, successfully combats numerous plant diseases *in situ*. *T. guizhouense* NJAU 4742 can suppress and kill the causative agent of banana wild disease *Fusarium oxysporum* f. sp. *cubense* 4 (Foc4), an ability not yet reported for other *Trichoderma* biocontrol agents. In order to learn about the genes involved in this property, we used T-DNA insertional mutagenesis and screened for mutants of *T. guizhouense* NJAU 4742 defective in mycoparasitism of Foc4. From a library of > 400 mutants, one was unable to overgrow Foc4 and had lost the ability to antagonize several other fungi. Using HiTAIL-PCR, the T-DNA was found to have integrated in this mutant into the terminator of a neutral metalloprotease gene of the MEROPS family M35, which was named *nmp1*. Consequently, the mutant was unable to express this gene, but this defect and the ability to antagonize and prey other fungi was rescued by retransformation with the wild-type *nmp1* gene. NMP1 was overproduced in *P. pastoris*, purified and shown to be processed to an active 18.7 kDa protein. NMP1 inhibited the growth of other fungi *in vitro*. *Nmp1* expression is induced by the presence of the other fungi including confrontations to itself, but its expression after physical contact depends on the mode of interaction: it increases in cases of biotrophic parasitism (e.g. *Botrytis cinera* and *Sclerotinia sclerotiorum*), while it decreases in cases of necrotrophic mycoparasitism (or predation) where the prey fungus has already died due to the attack of *T. guizhouense* (Foc4, *Alternaria alternata*). We conclude that NMP1 has major importance for the interaction of *T. guizhouense* with other fungi.

375. Increasing intracellular fatty acid levels of the filamentous fungus *Aspergillus carbonarius* ITEM5010 by regulating fatty acid catabolism. Malavika Sinha[1], Annette Sørensen[1,3], Kenneth S. Bruno[2], Istvan Weyda[3], Birgitte K. Ahring[1]. 1) Bioproducts, Sciences and Engineering Laboratory, Washington State University, Richland, WA; 2) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, Richland, WA; 3) Section for Sustainable Biotechnology, Aalborg University Copenhagen, Copenhagen SV, Denmark.

Biodiesel production by microorganisms has received much attention is the past few decades. Here, we report disrupting genes involved in the intracellular fatty acid and triglyceride degradation as means to increase the availability of these potential fuels for future biofuel production. Orthologs of the *farA* and *farB* genes involved in the beta-oxidation pathway in *Aspergillus nidulans* have been identified and disrupted in *Aspergillus carbonarius* ITEM 5010. The knockout mutants demonstrated loss of ability to grow on various long and short chain fatty acids. We demonstrate that by disrupting the *farA* and *farB* genes we successfully increased the levels of fatty acids and triglycerides in *A. carbonarius*. The disruption of the *farA* gene increased the fatty acids and triglyceride levels by 4-fold and 1.8-fold, respectively, while the deletion of the *farB* gene increased the fatty acids and triglyceride levels by 3-fold and 1.13 fold respectively. Fatty acid methyl esters (FAMES) analysis showed 1.3 fold increases in *farA* deletion mutants, however, no change was noticed in *farB* deletion mutants. Our data demonstrate that a simple deletion of fatty acid degradation genes can increase the amounts of fatty acids and triglycerides.

376. Non-transcription factor proteins regulate the enzyme synthesis in *Trichoderma reesei*. Y. Jiang, G. Zou, R. Liu, L. Chen, Z. Zhou. Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences,Shanghai 200032, China.

Trichoderma reesei is by far the preferred organism to produce cellulases in industry. To further improve its capacity to synthesize and secrete (hemi) cellulase enzymes, researchers have been paying close attention to the regulatory system for synthesis and secretory pathway of (hemi) cellulases in *T. reesei*. In this study, a non-transcription factor CelR, which is predicted as a protein, was found to be involved in (hemi) cellulase producing. The concentration of extracellular protein increased significantly in culture filtrates of the Qm9414 mutant (Δ*celR*Qm9414) with CelR deleted. The CMCase activity of Δ*celR*Qm9414 increased more than 200% and 70% in inducing medium (containing Avicel or lactose) and in repressing medium (containing glucose), respectively. Moreover, CelR was confirmed to be involved in transporting and hydrolyzation of lactose. The activity of extracellular galactosidase of Δ*celR*Qm9414 decrease 80% compared with that of wild Qm9414. By contrary, all the transcription level of the potential lactose permeases increased 88%~480% after CelR was deleted. As a negative regulatory protein, CelR can be applied to strain improvement. For example, the deletion of CelR in a β-glucosidase hyper-producing strain resulted in 30% increase of pNPGase activity, while 50% increase of pNPGase activity was observed with the combination of over-expression of the transcription factor Xyr1 and CelR deletion.

377. The detection and statistical analysis of alternative splicing variants in fungal genomes from RNA-Seq data. Thies Gehrmann[1], Jordi Pelkmans[2], Luis Lugones[2], Han Wösten[2], Marcel Reinders[1]. 1) Delft Bioinformatics Laboratory, Intelligent Systems, Delft University of Technology, Mekelweg 4, 2628 CD Delft, The Netherlands; 2) Department of Microbiology, Kluyver Centre for Genomics of Industrial Fermentation - Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Alternative splicing is a powerful tool in the proteomic repertoire of organisms. Studying alternative splicing thus gives great insight into cellular responses to changing environments. In fungi however, this study is hampered due to their dense genomes resulting in overlapping UnTranslated Regions (UTRs) of neighboring genes. Existing statistical and de-novo isoform analysis methods on the basis of next generation RNA sequencing reads do not account for this gene density and fuse neighboring transcripts together, making an analysis of

alternative splicing useless.
We propose a transformation of the genome that removes the influence of UTR overlaps by separating genes from each other. A pipeline that maps reads to the transformed genome then minimizes the number of unmappable reads due to the transformation through an iterative procedure. Based on the mapped reads existing RNA transcript reconstruction methods can be used that predict novel splicing variants from RNA-Seq data on this transformed genome.
We predicted alternative splicing variants in the basidiomycete Schizophyllum commune on a genome wide scale. We did so over an aggregate of different developmental stages and under various perturbations from RNA-Seq data to fully capture the flexibility of the genome to produce RNA products under different conditions. The method characterizes alternative splicing events found for 50% of the 16,000 genes in S. commune, including 2,000 isoforms with skipped exons, 3,000 splicing variants containing intron retentions, 900 introducing novel exons, 5,500 with 5' and 6,500 with 3' alternative splicing sites. We find that exons further downstream from the transcription start site of a gene are preferentially skipped, but that exon skipping is less common than intron retentions. 5' and 3' alternative splicing events that generally shorten exons were less common. Taken together these results show that the S. commune transcriptome is more complex than previously known.

378. Analysis of an *nsdC* mutant in *Aspergillus flavus* reveals an extensive role in the regulation of secondary metabolic gene clusters. Matthew Gilbert, Brian Mack, Darlene Downey, Deepak Bhatnagar, Jeffrey Cary. Food and Feed Safety Unit, United States Department of Agriculture, New Orleans, LA.

Aspergillus flavus is a saprophytic fungus that can invade and contaminate agronomically important crops. The fungus produces a number of toxic secondary metabolites, such as aflatoxin, which are synthesized from genes located in close proximity with each other on the chromosome. *A. flavus* has approximately 55 such gene clusters. NsdC is a C_2H_2-type transcription factor that has been shown to play a role in asexual development and aflatoxin production in this fungus. Previous data demonstrated that *nsdC* knockout mutants in the sclerotial L- morphotype *A. flavus* CA14 exhibited perturbed development of conidiophores, altered colony pigmentation and loss of sclerotia and aflatoxin production. To better determine the role of *nsdC* in the regulation of secondary metabolite production in *A. flavus* we conducted RNA-sequencing analysis using an *nsdC* knockout in the S-morphotype *A. flavus* SRRC 70 (Af70). These studies, combined with SMURF analysis, revealed that 83% of predicted secondary metabolic gene clusters had altered expression profiles in the *nsdC* knockout strain. Expression of genes in clusters responsible for penicillin, asparasone, ustiloxin B, and piperazine production were increased, whereas, genes responsible for aflatrem, aflatoxin, and kojic acid were decreased compared to that of the control. HPLC-MS analysis of fungal extracts showed decreased levels of the aflatrem metabolite present in the knockout strain corresponding with the gene expression data. Our analysis also revealed significant down regulation of a putative secondary metabolic gene cluster that is present in Af70 but not in found in other *A. flavus* isolates including the type strain NRRL 3357. This cluster contains genes predicted to encode a polyketide synthase, two p450s, a Zn(2)–Cys(6) transcription factor and other decorating enzymes. Analysis of these secondary metabolic gene profiles with special emphasis on the functional characterization of the novel gene cluster in Af70 will be discussed.

379. Comparative transcriptomics of the human pathogen *Histoplasma* reveals conserved and widespread re-programming of transcript length. Sarah Gilmore, Mark Voorhies, Anita Sil. Department of Microbiology & Immunology, University of California, San Francisco, San Francisco, CA.

Eukaryotic cells integrate many layers of gene regulation beyond the initial transcription of a gene to coordinate complex cellular processes such as embryogenesis and cellular development; however mechanism of post-transcriptional gene regulation lag behind our understanding of transcriptional regulatory events. The thermally dimorphic human fungal pathogen *Histoplasma capsulatum* (*Hc*) exhibits readily reversible unicellular (budding yeast) and multicellular (hyphae) developmental states that are controlled by the environmental cue of temperature. Thus *Hc* represents an ideal organism to probe fundamental questions regarding the basic mechanisms of gene regulation that eukaryotic cells employ to cue multicellular development. In this work, we use the developmentally distinct cells types of *Hc* to uncover mechanisms of post-transcriptional gene regulation during development. Employing recent advances in RNA sequencing, de novo transcriptome reconstruction methodologies, and ribosome profiling (which measures translational efficiency genome-wide), we uncovered a novel means of post-transcriptional gene regulation in the yeast and hyphal cell types of *Hc*. Remarkably, we find that ~2% percent of the *Hc* genome exhibits differential 5' transcript ends (or leader sequences) between the two morphogenetic states. Comparative transcriptomics analyses of RNA sequencing data across multiple *Hc* lineages indicates that the majority of differential leader transcript architecture is conserved, suggesting that 5' transcript extensions are a non-random, biologically regulated process. Ribosome and mRNA density measurements uncovered a class of these longer leader transcripts that exhibit tight transcriptional and translational regulation. Further examination of this group of transcriptionally and translationally regulated genes reveals that some are involved in controlling *Hc* morphology and that their strict regulation may be necessary for the organism to make appropriate developmental decisions.

380. Role of laccases of *Schizophyllum commune* in black slate degradation. J. Kirtzel[1], S. Madhavan[1], M. Gube[2], E. Kothe[1]. 1) Microbial Communication, Friedrich Schiller University, Jena, Germany; 2) Soil Science of Temperate Ecosystems, Georg August University, Goettingen, Germany.

The basidiomycete *Schizophyllum commune* has been in the focus of research for many decades. Because of its short life cycle, it has become a model organism for physiological and genetic studies. As a white-rot fungus, *S. commune* excretes enzymes which are responsible for the decomposition of lignin and other organic compounds from wood. Recent studies showed the involvement of this basidiomycete in low grade metamorphic rock decay. Due to a wide substrate specificity of some of these enzymes, it is supposed that they also mediate black slate bioweathering. Laccases are multi-copper oxidases that catalyze the oxidation of a wide variety of organic and inorganic substrates. It was assumed that excreted laccases are taking part in this process and effect the carbon mobilization from black slates. In the genome of *S. commune*, two laccases and four laccase-like genes have been identified. In order to gain insights into the role of laccases in black slate degradation, an overexpression of *lcc2* has been performed. A quantitative real-time PCR is performed to analyze the

expression changes at transcript level. Growth experiments are going to show whether laccase over expressing strains show enhanced black slate degradation.

381. A novel RNase III-like protein participates in an RdRP-dependent Dicer-independent degradation mechanism of endogenous mRNAs in basal fungi. Trung A Trieu[1], Silvia Calo[1], Francisco E Nicolas[1], Simon Moxon[2], Tamas Dalmay[3], Santiago Torres-Martínez[1], Victoriano Garre[1], Rosa M Ruiz-Vazquez[1]. 1) Dept Genetics and Microbiology, Univ Murcia, Murcia, Spain; 2) School of Computing Sciences, Univ East Anglia, Norwich, UK; 3) School of Biological Sciences, Univ East Anglia, Norwich, UK.

The canonical RNA silencing (RNAi) mechanism requires the presence of the RNase III Dicer to process double-stranded RNA (dsRNA) precursors into small interfering RNAs (siRNAs), and an Argonaute endonuclease that binds siRNAs and uses them as a guide to identify and cleave complementary target mRNA. In the basal fungus *Mucor circinelloides*, the RNAi pathway also requires RNA-dependent RNA polymerase (RdRP) enzymes to produce dsRNA from single-stranded RNA inducers or to amplify siRNA signals. The canonical *dicer*-dependent RNAi pathway in *Mucor* produces endogenous small RNAs derived from exons (ex-siRNAs) that regulate the expression of protein-coding genes. But, besides this canonical pathway, *Mucor* presents a non-canonical RNAi mechanism that generates a new *rdrp*-dependent *dicer*-independent ex-siRNA class. Analysis of these ex-siRNAs reveals that they are produced by a degradation pathway in which participates the RdRP-1 and/or RdRP-2 proteins to signal specific transcripts that have to be degraded by a previously unknown RNase. This non-canonical pathway regulates specific cellular processes, such as sexual interaction and the response to environmental stresses. Searching the *Mucor* genome for candidate RNases and functional analysis identified a new protein, named R2D2, involved in the *rdrp*-dependent *dicer*-independent pathway. This RNase presents unique domain architecture, it is only found in basal fungi and it is also involved in the canonical *dicer*-dependent silencing pathway, highlighting its crucial role in the biogenesis and function of regulatory ex-siRNAs. The involvement of RdRPs in the RNA degradation process could represent the first step in the evolution of RNAi and may signal a genetic link between mRNA degradation and post-transcriptional gene silencing.

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382. Functional characterization of *rmtA*, a gene encoding a putative arginine methyltransferase, in the opportunistic plant pathogen *Aspergillus flavus*. Tim Satterlee[1], Yanbin Yin[1], Jeffery W. Cary[2], Deepak Bhatnagar[2], William C. Nierman[3], Liliana Losada[3], Ana Calvo[1]. 1) Department of Biological Sciences, Northern Illinois University, DeKalb, IL; 2) Food and Feed Safety Research Unit, USDA/ARS, Southern Regional Research Center, New Orleans, LA; 3) Infectious Diseases Program, J. Craig Venter Institute, Rockville, MD.

The genus Aspergillus is a group of filamentous fungi with representatives that are beneficial as well as detrimental to humans. Among them, Aspergillus flavus is a well-known opportunistic pathogen often found colonizing food commodities both pre- and post-harvest, particularly oilseed crops. During growth on corn, peanut, treenuts and cottonseed, A. flavus produces highly cytotoxic and carcinogenic mycotoxins, such as aflatoxins. In order to gain insight into the genetic regulatory mechanisms controlling A. flavus mycotoxin production, as well as dissemination and survival we characterized rmtA, a gene encoding a type I arginine methyltransferase involved in histone modifications. Aflatoxin production is significantly reduced in a rmtA deletion mutant (ΔrmtA), and produced at significantly higher levels in a rmtA over-expression strain (OErmtA) compared to the wild type, indicating that rmtA is a positive regulator of aflatoxin biosynthesis. The production of other unknown compounds also presented a similar pattern, suggesting a broader regulatory effect for rmtA in secondary metabolism. Interestingly, ΔrmtA colonies hypercondiate, suggesting that rmtA is a repressor of asexual development in A. flavus. Furthermore, sclerotial production is also rmtA-dependent. In the absence of rmtA, sclerotial development is significantly reduced or prevented, while these survival structures are produced at greater numbers in the OErmtA strain compared to wild type. Genome database searches using the RmtA deduced amino acid sequence revealed high conservation among filamentous fungi, as well as with other eukaryotes. Transcriptome analysis revealed that rmtA controls the expression of numerous genes involved in development and secondary metabolism, including those required for aflatoxin, cyclopiazonic acid and aflatrem production.

383. Genome-wide transcriptional regulation and chromatin dynamics in response to nitrogen availability in *Aspergillus nidulans*. Damien J. Downes[2], Zhengqiang Miao[1], Djordje Djordjevic[3], Vinita Deshpande[3], Ang Li[1], Kaeling Tan[1], Grethel Y Busot[2], Joshua W.K. Ho[3], Richard B. Todd[2], Koon Ho Wong[1]. 1) Faculty of Health Sciences, University of Macau, Taipa, Macau; 2) Department of Plant Pathology, Kansas State University, Manhattan KS, USA; 3) Victor Chang Cardiac Research Institute, and the University of New South Wales, Sydney, NSW, Australia.

Fungi adapt their metabolism to nitrogen nutrient availability primarily via global transcriptional control of nitrogen uptake and metabolic genes. In *Aspergillus nidulans*, the GATA transcription factor AreA activates genes for nitrogen metabolism in response to nitrogen limitation or nitrogen starvation. We have performed ChIP-seq of RNA polymerase II in wild type and an *areA*Δ mutant in the presence of ammonium and during nitrogen starvation to identify the genome-wide AreA-dependent gene expression program. Using ChIP-seq of HA-epitope-tagged AreA, we determined the pan-genomic direct targets of this global regulator. We have also mapped global chromatin dynamics and genome-wide chromatin modifications in response to nitrogen nutrient availability. Our data reveal nitrogen transport, metabolic and regulatory gene targets as well as new targets for AreA, including promoters of iron siderophore biosynthesis genes, heme metabolism genes, and secondary metabolism genes. Differential chromatin modifications occurred in response to nitrogen availability and were enriched in secondary metabolism genes.

384. Interrelation between Ras and Phosphatidylinositol signaling in the basidiomycete *Schizophyllum commune*. R. Murry, E. Kothe. Microbial Communication, Friedrich Schiller University, Jena, Germany.

Ras signaling pathway was intensively studied in *Schizophyllum commune*. The small Ras protein is known to be involved in growth rate, hyphal branching and growth orientation, nuclear migration, clamps fusion, basidiospore production, fruiting body morphology, as well as

meiosis. On the other hand, the function of Inositol monophosphatase has not been explored yet. However, Inositol monophosphatase is known to be regulated in a Ras dependent manner. This phenomenon tantalizes the question of crosstalk occuring between IMPase and Ras.

IMPase is a pivotal enzyme in phosphatidylinositol (PI) signaling by dephosphorylation of inositol monophosphate to produce inositol, which is thereafter utilized to form phosphatidylinositol (PI). IMPase is specifically inhibited by lithium which is known as inositol depletion hypothesis. The presence of lithium resulted fungal growth reduction and multihyphal branching and induced the *imp* gene expression in the wild type, whereas the opposite effect on gene expression was shown in the constitutivly active Ras1 mutant. On the other hand, lithium chloride inhibits the IMPase activity as a compensatory effect of *imp* induction and the same effect of IMPase reduction was observed in a Ras dependent manner.

Genetic manipulation resulting in a loss of IMPase function by homologous recombination is performed to study the cellular effects in more detail. In addition, proteome analysis, as well as inositol and inositol phosphate measurement will be performed to gain closer to the insights into the crosstalk between Ras and PI signaling.

385. *MoJMJ1*, a histone demethylase gene encoding JmjC domain is required for pathogenic development of the rice blast fungus, *Magnaporthe oryzae*. A. Huh[1], S. Kwon[1], S. Kim[1], J. Jeon[1], Y.-H. Lee[1,2]. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, South Korea; 2) 2Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea.

Histone methylation plays important roles in regulating chromatin dynamics and transcription. It was shown that disruption of histone methylation could lead to developmental defects and cancer in plants and mammals, respectively. Despite the generality of histone modifications as epigenetic mechanism in eukaryotes, implication of histone modifications in fungal pathogenesis is beginning to emerge. Here we report identification and functional analysis of a putative JmjC-domain containing histone demethylase in a model plant pathogenic fungus, *Magnaporthe oryzae*. Among seven genes encoding putative JmjC-domain containing histone demethylase identified in the fungus, deletion of *MoJMJ1*, belonging to JARID group, resulted in defects in vegetative growth, asexual reproduction, autolysis, appressorium formation and invasive growth. Western blot analysis showed that overall H3K4me3 level increased in the deletion mutant, compared to wild-type strain, suggesting histone demethylase activity of MoJMJ1. Introduction of native *MoJMJ1* gene into *ΔMojmj1* restored not all but defects in vegetative growth, asexual reproduction, autolysis and appressorium formation, indicating the importance of regulating histone demethylation through *MoJMJ1* during fungal development and appressorium formation. Comparative analysis of RNA-seq experiments for the mutant and a complementation strain suggested that transcriptional regulation of some of the known pathogenicity genes such as CON7 is dependent on *MoJMJ1*. RNA-seq analysis also revealed enrichment of *MoJMJ1*-dependent genes in functional categories involved in interaction with host species. Our work on *MoJMJ1* provides insight into H3K4me3-mediated regulation of transcriptome and infection-specific development in a plant pathogenic fungus.

386. Thioredoxins are essential for appressorium formation, conidiation, and circadian rhythm in *Magnaporthe oryzae*. C Jiang[1,2], SJ Zhang[2], JR Xu[1,2]. 1) Purdue University, West lafayette, IN; 2) Northwestern A&F University, Yangling, China.

In the rice blast fungus *Magnaporthe oryzae*, the Mst11-Mst7-Pmk1 MAP kinase (MAPK) pathway is essential for appressorium formation and infectious growth. Because the redox status can affect the dimerization of MAPK kinases and activation of downstream MAP kinases, in this study we isolated and characterized the two thioredoxin genes, *TRX1* and *TRX2*, in *M. oryzae*. *TRX2* had a higher expression level and was found to be the predominant thioredoxin gene. Whereas the *trx1* mutants had no detectable phenotypes except a minor reduction in conidiation, the *trx2* mutant rarely produced conidia and was down-regulated in the expression of *COM1*, *HTF1*, and *CON7* transcription factor genes. It was also significantly reduced in growth rate, aerial hyphal growth, and appressorium formation, and only caused rare, non-typical lesions on rice seedlings. However, the *trx1 trx2* double mutant was blocked in conidiation and conidiophore development and it was non-pathogenic in infection assays with hyphal fragments or culture blocks. Germ tubes and hyphal tips of the *trx2* and *trx1 trx2* mutants were defective in appressorium formation and failed to penetrate and grow invasively in plant cells. In the *trx2* and *trx1 trx2* mutants, the phosphorylation level of Pmk1 but not Mps1, was significantly reduced. Western blot analysis indicated that deletion of *TRX2* affected proper folding or dimerization of the Mst7 MEK kinase that functions upstream from Pmk1. Site-specific mutagenesis indicated that Cys305 of Mst7 is important for its function. Therefore, *TRX2* may affect appressorium formation and invasive growth via Pmk1. In addition, we found that *TRX2* also plays a role in responses to oxidative stress, accumulation of intracellular ROS, and circadian rhythm. Taken together, our data showed that the thioredoxin genes play important roles in intracellular ROS signaling, conidiogenesis, hyphal growth, and pathogenesis in *M. oryzae*.

387. Infection structure-specific expression of lipase-like effector supports appressorial functionality and fungal cell-to-cell colonization of the rice blast fungus, *Magnaporthe oryzae*. E Oliveira-Garcia, B Valent. Plant Pathology, KSU, Manhattan, KS.

Rice blast caused by *Magnaporthe oryzae*, a hemibiotroph and facultative pathogen, is the most important disease of rice worldwide. At the early stage of infection, the germ-tube differentiates to form an appressorium immediately adjacent to the conidium. The appressorium serves for the direct penetration of the host. After host penetration, *M. oryzae* establishes a biotrophic interaction. It is assumed that different strategies employed by the fungus to avoid triggering defense responses, including masking of invading hyphae or active suppression of host defense mechanisms, are essential for a biotrophic parasitic lifestyle. During the infection process, *M. oryzae* secretes various effectors, which are hypothesized to be involved in effective host infection. To date, little is known about the influence of lipases during infection of plants by fungi. Here, we show that a lipase-like effector is up-regulated during plant penetration and biotrophic development. Using fluorescent protein tagging, we found lipases localized to stage-specific compartments at the host-pathogen interface.

Importantly, we show that this lipase is focally secreted from the appressorial penetration pore into the O-ring before host invasion, revealing new levels of functional complexity for this fungal organ. Furthermore, we demonstrate that lipase-like effector accumulates massively in plant cell wall crossing points during cell-to-cell colonization by the fungus. This accumulation of the lipase-like effector at crossing points was observed during invasion of four consecutive rice cells following initial successful colonization of the first cell by the fungus. Based on these results we conclude that infection structure-specific expression of lipase-like effector supports appressorial functionality and/ or fungal cell-to-cell colonization. Our results also suggest a potential role of lipases for manipulation host cell channels, plasmodesmata, by the fungus.

388. Two conserved phosphorylation sites of the *Candida albicans* Hog1 protein are important for white-opaque switching, mating response and pheromone-stimulated cell adhesion. Wen-Han Chang, Fu-Sheng Deng, Shen-Huan Liang, Ching-Hsuan Lin. Department of Biochemical Science & Technology, National Taiwan University, Taipei, Taiwan.

Candida albicans is an opportunistic human fungal pathogen and able to cause life-threatening infections in immunocompromised patients. *C. albicans* has an unique morphological transition between white and opaque phases. These two cell forms have different properties in virulence, mating capability, biofilm formation and host-cell interaction. White-opaque transition is regulated by several external stimuli, such as CO_2, N-acetylglucosamine and oxidative stress. Our previous study revealed that deletion of *SSK2*, *PBS2* or *HOG1* gene resulted in 100% white-to-opaque switching on SC medium, and suppressed the mating response. Hog1 protein has two important phosphoacceptors, Thr-174 and Tyr-176, and will be activated when phosphorylated in response to stimuli. In this study, we first demonstrated that two conserved phosphorylation sites are not only required for stress response, but also involved in white-opaque switching, mating and pheromone-stimulated cell adhesion. Six Hog1 point-mutated strains were generated, including non-phosphorylated strains (*HOG1*^{T174A}, *HOG1*^{Y176F} and *HOG1*^{T174A,Y176F}) and mimic phosphorylated strains (*HOG1*^{T174D}, *HOG1*^{Y176D} and *HOG1*^{T174D,Y176D}). Point mutation on Thr-174, Tyr-176 or in combination of Hog1 protein in *MTL* homozygous strains of *C. albicans* would stimulate opaque cell formation in a frequency of 100% on SC medium. Similar to the *hog1* mutant, all of the point mutation strains were sensitive to both osmotic and oxidative stresses. Western blotting showed that Hog1 proteins were expressed in these mutants, but could not be phosphorylated when treated with H_2O_2. Furthermore, mating projections of point-mutated strains were significantly shorter and their mating efficiencies were lower than those of the wild-type. In addition, numbers of pheromone-induced cell adhesion of white cells in all point-mutated strains reduced. Taken together, our study demonstrated that mutation on either Thr-174 or Tyr-176 of Hog1 resulted in similar characters with *hog1* mutants in white-opaque transition, sexual mating and pheromone-induced cell adhesion in *C. albicans*.

389. A GATA transcription factor in *Blastomyces dermatitidis* regulates morphology, iron homeostasis and lipid metabolism. A. Marty[1], A. Broman[2], R. Zarnowski[1], T. Dwyer[1], L. Bond[3], A. Lounes-Hadj Sahraoui[4], J. Fontaine[4], J. Ntambi[3,5], C. Kendziorski[2], G. Gauthier[1]. 1) Medicine, University of Wisconsin - Madison, Madison, WI; 2) Department of Biostatistics and Medical Informatics, University of Wisconsin - Madison; 3) Department of Biochemistry, University of Wisconsin - Madison; 4) Université du Littoral Côte d'Opale, Unité de Chimie Environnementale at Interactions sur le Vivant, Calais, France; 5) Department of Nutritional Sciences University of Wisconsin - Madison.

Blastomyces dermatitidis belongs to a group of fungi that adapt to temperature by converting between yeast (37°C) and mold (22°C). Knowledge about the molecular mechanisms underlying this morphologic switch in response to temperature remains limited. In *B. dermatitidis*, we identified a GATA transcription factor, *SREB*, which is important for the transition to mold. Null mutants (*SREB*Δ) fail to complete the conversion to mold and cannot properly regulate siderophore biosynthesis. Gene expression microarrays were used to compare *SREB*Δ to an isogenic wild type isolate during the early part of the morphologic switch (0 - 48 hours). LIMMA and EBarrays analyses demonstrated that deletion of *SREB* affected transcription of 11.6 - 19.9% genes in the *B. dermatitidis* genome across the time course. Bioinformatic and biochemical analyses indicated *SREB* was involved in diverse processes including the biosynthesis of triacylglycerol, ergosterol, and lipid droplets. Integration of microarray and chromatin immunoprecipitation data with real-time PCR (ChIP-qPCR) identified a subset of genes bound and regulated by *SREB in vivo* as yeast (37°C) and during the switch to mold (22°C). This included genes involved with iron homeostasis as well as genes unrelated to iron assimilation. Functional analysis demonstrated *SREB* bound and negatively regulated a bZIP transcription factor. Overexpression of the bZIP transcription factor resulted in a similar morphologic phenotype as *SREB*Δ - impaired conversion to mold at 22°C. In conclusion, *SREB* functions as a regulator of transcription at 37°C and 22°C to affect disparate processes including morphogenesis, iron homeostasis, and lipid metabolism.

390. Circadian clock-dependent glycogen metabolism in *Neurospora crassa*. M. Baek[1], A. Dovzhenok[2], S. Lim[2], C. Hong[1]. 1) Department of Molecular and Cellular Physiology, College of Medicine, University of Cincinnati, Cincinnati, OH 45267; 2) Department of Mathematical Sciences, University of Cincinnati, Cincinnati OH 45221.

The molecular machinery of circadian rhythms maintains robust cell-autonomous oscillations with a period of about 24 hours, and provide temporal information to other physiological processes to anticipate timing of activity/resting. Interlocked transcriptional-translational feedback loops govern circadian rhythms in *Neurospora crassa*. In this report, we investigate functional roles of circadian rhythms in glycogen metabolism in *N. crassa* with mathematical modeling by constructing a model of circadian rhythms and glycogen metabolisms as an interconnected network. Our mathematical model predicts in phase circadian oscillations of glycogen metabolic gene expression, *glycogen synthase 1 (gsy-1*) and *glycogen phosphorylase 1 (gph-1*), which are involved in glycogen synthesis and glycogen breakdown, respectively, and loss of their oscillations in circadian arrhythmic mutants. Then, we experimentally validate rhythmic expression *gsy-1* and *gph-1*. Circadian oscillations of *gsy-1* and *gph-1* gene expression are abolished in circadian arrhythmic mutants, *wc-1*^{ko} or *frq*^{ko}. Intriguingly, we observe reduced amplitude oscillations of *gsy-1* and *gph-1* gene expression in *csp-1*^{ko}, which is a WCC-regulated transcriptional repressor. Collectively, we observe that the circadian clock regulates rhythmic process of glycogen metabolism.

391. Complex formation of RNA silencing proteins in the perinuclear region of *Neurospora crassa*. Logan Decker[1], Erin Boone[1], Hua Xiao[1], Benjamin Shanker[1], Shannon Boone[1], Shanika Kingston[2], Seung Lee[1], Thomas Hammond[3], Patrick Shiu[1]. 1) Biological Sciences, University of Missouri, Columbia, MO; 2) Department of Biology, Barry University, Miami Shores, FL; 3) School of Biological Sciences, Illinois State University, Normal, IL.

The filamentous fungus *Neurospora crassa* is made up of interconnected cells where nuclei and other cellular components share a common cytoplasm. This trait, while beneficial for distributing resources, may promote the spread of detrimental elements such as transposons and viruses. Perhaps for this reason, *Neurospora* possesses several surveillance mechanisms that operate during different phases of its lifecycle. One of these defense mechanisms is known as meiotic silencing by unpaired DNA (MSUD). In MSUD, genes not paired during meiosis are targeted by a post-transcriptional gene silencing pathway. Here, our bimolecular fluorescence complementation (BiFC) study suggests that common RNAi proteins (RNA-directed RNA polymerase, Dicer, and Argonaute) as well as others form a meiotic silencing complex in the perinuclear region (the "surveillance checkpoint"), with intimate interactions among the majority of them. We have also shown that SAD-2 (a putative scaffold protein) is likely the anchor for this assembly.

392. Insights into the role of the CRE-1 transcription factor in the circadian clock regulation of the metabolism of cellulose in Neurospora crassa. Rodrigo Díaz-Choque, Luis F Larrondo. 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 by enabling processes to occur at appropriate times of the day. In the model organism *Neurospora crassa*, ~20% of its genes are under circadian control and interestingly; many of them are related to several metabolic processes such as cellulose degradation. However, the pathways involved in relaying the time-of-day information to the expression of the cellulolytic genes, remains unclear.

We are interested in elucidating the transcriptional mechanisms interconnecting circadian and metabolic processes, particularly regarding organismal fitness. Thus, we are analyzing carbon catabolite repression (CCR) and cellulolytic capabilities in a circadian context, through the evaluation of the circadian role of CRE-1, a crucial metabolic transcription factor involved in both processes aforementioned.

Through transcriptional fusion reporters and global expression analysis (RNA-seq), we found evidence that suggests that this transcription factor acts as a link between the circadian and metabolic pathways. These data provide insights on how the circadian clock is influencing Neurospora physiology, potentially impacting a biotechnological relevant process like biomass conversion.

393. The bZIP transcription factor HAC-1 is involved in the unfolded protein response and is necessary for efficient plant cell wall deconstruction in *Neurospora crassa*. Alejandra Goity, Alejandro Montenegro-Montero, Luis F. Larrondo. Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Excessive accumulation of unfolded proteins inside the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR), a stress pathway conserved from yeast to mammals, and that is crucial for the maintenance of ER homeostasis during protein synthesis, folding and secretion in eukaryotic cells. Due to the high protein secretory capacity displayed by filamentous fungi the study of this pathway has gained special interest. The bZIP transcription factor HAC-1 is the main regulator that controls the expression of several genes required during the UPR. In this work, we identified and characterized the *hac-1* gene in the filamentous fungus *Neurospora crassa*. We show that its mRNA undergoes an ER stress-dependent unconventional splicing reaction, which in *N. crassa* removes a 23 nt intron and leads to a change in the open reading frame. By disrupting the *N. crassa hac-1* gene, we determined it to be crucial for activating UPR and for proper growth in the presence of ER stress-inducing chemical agents. Neurospora naturally grows on dead plant material, composed primarily by lignocellulose, and is a model organism for the study of plant cell wall deconstruction, a process that involves the secretion of multiple enzymes to the extracellular medium. Notably, we found that *hac-1* is specifically required for growth on cellulose and that growth on this carbon source appears to impose major demands on ER function, thus broadening the range of physiological functions of the UPR in filamentous fungi. The characterization of this signaling pathway in *N. crassa* will help in the study of plant cell wall deconstruction by fungi and its manipulation may result in important industrial biotechnological applications. CONICYT, GO-21120421, FONDECYT 1131030, MN-FISB NC120043.

394. Identification of Neurospora Shelterin. Miki Uesaka, Ayumi Yokoyama, Shinji Honda. Life Science Unit, University of Fukui, Eiheiji, Fukui, Japan.

A telomere-specific protein complex, Shelterin, caps and protects chromosome ends against inappropriate DNA damage, telomeric fusion and telomere length in eukaryotes. However, in any filamentous fungi, the corresponding Shelterin has not been identified. Here we show Neurospora Shelterin which is composed of at least five proteins. The two components, POT-1 and RAP-1, are conserved from yeasts to mammals whereas the others are only conserved in Ascomycota. By Chromatin Immunoprecipitation (ChIP) assays, we confirmed that one of Neurospora Shelterin components is specifically localized to telomeres. Fluorescence microscopic analyses revealed that all the components of Neurospora Shelterin are co-localized to 2~4 foci within nuclei. The telomeric foci are mostly associated with nuclear envelope and heterochromatin but not a single centromeric spot. Furthermore, the localization is not dependent of H3K9 methylation that directs heterochromatin and DNA methylation.

395. Discovering complex carbohydrate sensing pathways in the filamentous fungus *Neurospora crassa*. Lori B. Huberman, N. Louise Glass. UC Berkeley, Berkeley, CA.

Identifying and utilizing the nutrients available in the most efficient manner is a challenge common to all organisms. In humans inaccurate or failed nutrient sensing can result in a variety of diseases including diabetes and obesity, and cancer progression has been shown to rely on increased glucose uptake and changes in nutrient sensing. Many of the nutrient sensing pathways are conserved from yeast to humans, and studies on nutrient sensing in unicellular eukaryotes have been instrumental in elucidating nutrient sensing pathways in humans. Unlike budding yeast, the model filamentous fungus, *Neurospora crassa*, is capable of utilizing a variety of carbohydrates: from simple sugars to the complex sugar chains found in plant cell walls. In order to efficiently exploit the available resources, *N. crassa* must be capable of sensing and responding to the presence of these different carbohydrates. Several transcription factors have been identified that activate the transcription of plant cell wall-degrading enzymes including XLR1, which is responsible for activating transcription of hemicellulases in the presence of xylan, and CLR1, which is responsible for activating transcription of cellulases in the presence of cellulose. However, expression of these transcription factors in the absence of an inducer does not lead to transcriptional activation of their target genes, indicating that activation or derepression of XLR1 and CLR1 is necessary. We performed a screen of the *N. crassa* deletion collection to identify mutants that are unable to properly regulate the transcription of cellulases and hemicellulases in the presence of cellulose and xylan, respectively, and identified several genes which are predicted to be involved in metabolic processes. As we continue to characterize these mutants, we expect to shed light on the xylan and cellulose sensing pathways in *N. crassa* and, more generally, provide insight into nutrient sensing in other fungi and multicellular eukaryotes.

396. Understanding the Circadian Output Gene Regulatory Network using the Clock-Controlled Transcription Factor ADV-1 in *Neurospora crassa*. Oneida Ibarra[1], Rigzin Dekhang[1], Elham Aziz[2], Cheng Wu[1], Kristina Smith[3], Jill Emerson[4], Jay Dunlap[4], Michael Freitag[3], Matthew Sachs[1], James Galagan[2], Deborah Bell-Pedersen[1]. 1) Texas A&N University, 3258 Tamu, Department of Biology, College Station, TX; 2) Boston University, 44 Cummington St., Department of Biomedical Engineering, Boston, MA; 3) Oregon State University, 3021 Agriculture and Life Sciences Building, Center for Genome Research and Biocomputing, Corvallis, OR; 4) Dartmouth College, Remsen Room 702, Geisel School of Medicine at Dartmouth, Hanover, NH.

Organisms keep track of time, and are synchronized to the 24-hr environmental cycle, using a molecular circadian clock. About 30% of the genome is controlled by the clock at the level of transcript abundance in eukaryotic cells, but the mechanisms by which the clock regulates rhythms and peak phase in mRNA levels are not well understood. Using Neurospora as a model organism to investigate the circadian output gene network, we identified direct targets of the core clock component and blue light photoreceptor WHITE-COLLAR Complex (WCC) using chromatin immunoprecipitation, followed by sequencing (ChIP-seq). The direct targets of the WCC were enriched for 24 first-tier transcription factors (TFs), suggesting that aspects of light regulation and circadian output pathways are hierarchical. ADV-1, one of the first-tier TFs, is robustly rhythmic, defective in clock-controlled development, and is closely linked to the downstream developmental and metabolic network. In addition to the WCC, there are several first-tier TFs, including ADV-1 itself, that bind to the promoter of *adv-1*. ADV-1 in turn, binds to the promoters of downstream TF genes, and genes involved in metabolism, cell fusion, and development. Not surprisingly, many of the downstream targets of ADV-1 are clock-controlled, but they peak at different times of the day. Using TF deletion strains, we are testing the prediction that the upstream and downstream TF network revolving around ADV-1 generates distinct temporal dynamics of gene expression critical to the coordination of rhythmic processes, including rhythmic metabolism and development.

397. Investigating the RNA molecules of meiotic silencing by unpaired DNA (MSUD). Dilini A. Samarajeewa[1], Nicholas A. Rhoades[1], Hua Xiao[2], Kevin A. Edwards[1], Patrick K.T. Shiu[2], Thomas M. Hammond[1]. 1) School of Biological Sciences, Illinois State University, Normal, Illinois, 61790; 2) Division of Biological Sciences, University of Missouri, Columbia, Missouri, 65211.

In *Neurospora crassa*, meiotic silencing by unpaired DNA (MSUD) is a process that detects and silences unpaired DNA between homologous chromosomes during sexual development. It is believed that MSUD works through an RNA interference-related pathway that begins with the production of aberrant RNAs (aRNAs). However, these aRNAs have yet to be identified. Here, we present results from experiments designed to identify these theoretical molecules. Additionally, we present results from our analysis of a novel MSUD protein. This protein has RNA binding domains and it could be involved in transporting MSUD-related RNA molecules, such as aRNAs, to their proper destination in the meiotic cell.

398. Grass to Gas: Discovering the Transcriptional Regulation networks of *Neurosprora crassa* Involved in Plant Cell Wall Degradation. Vincent Wu[1], David Kowbel[1], Igor Gregoriev[2], N. Louise Glass[1]. 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Department of Energy Joint Genome Institute, Walnut Creek, CA.

Conversion of cellulosic plant biomass to biofuels holds great potential for alleviating our reliance to fossil fuels. A major goal of this research is to develop fungi to be suitable for secreting large volumes of plant cell wall degrading enzymes (CWDEs). In order to productively direct our efforts, a solid understanding of how these fungi regulate the expression of CWDEs must first be established. Using *Neurospora crassa* as a model, we have begun to characterize how filamentous fungi respond to different carbon sources. By comparing transcriptional profiles of *N. crassa* grown on a variety of carbon sources, we have determined that the fungus responds uniquely to different polysaccharides. Furthermore, we have concluded that *N. crassa* uses simple sugar molecules as a way to sense what is in its environment. Unique CWDEs are transcriptionally upregulated in the presence of low concentrations of these simple sugar dimers and monomers. To move forward we are investigating predicted transcription factors that are upregulated in concert with different sets to CWDEs in order to determine if they are involved in regulating transcription of these CWDEs. Finding these transcription factors is key to understanding the regulatory pathways of CWDE expression and will establish the grounds from which we can conduct directed engineering of filamentous fungi for protein production.

399. H3K9 trimethylation at the upstream intergenic regions play a key role in regulating expression of *catalase-3* in *Neurospora crassa*. Wang Yajun, Gai Kexin, Dong Qing, Ding Zhaolan, He qun, Wang Ying. Microbiology and Immunology, College of Biology, CAU, Beijing, China.

H3K9 trimethylation at the upstream intergenic regions play a key role in regulating expression of *catalase-3* in *Neurospora crassa*

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Neurospora crassa, a filamentous fungus, is a powerful, genetically tractable system for studies of histone modifications and gene expression. To further understand the mechanism of histone modifications for gene regulation in fungus, we sought to identify pathways that selectively regulate the expression of special genes in *N. crassa*. Here we found that an upstream region was sufficient to regulate the expression of a catalase gene.The precise regulation of gene expression is essential for living organisms development and underlies the ability to respond to stimuli. In *N. crassa*, the expression of one catalase gene *cat-3* is tightly regulated during asexual development and under stress conditions of H_2O_2. Our studies revealed that CAT-3 protein levels increased in mutants impaired in proper H3K9 trimethylation. Sequence analysis revealed a 5-kb intergenic region adjacent to the *cat-3* promoter. ChIP assays exhibited that **ed in th**H3K4me3 and H3K9me3 were enriched at promoter and upstream intergenic regions, respectively. In addition, our data showed that the expression of CAT-3 was supresssed by H3K9me3 at the upstream region of cat-3 promoter. These results may suggest a conserved mode of enzyme regulation in fungi through chromatin modifications.

400. Genetics dissection of yeast-hyphal transition in the pathogenic zygomycete *Mucor circinelloides*: Calcineurin as a global fungal virulence factor. Soo Chan Lee. Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC.

Mucor circinelloides is a versatile zygomycete/basal fungal species. It is one of the etiological agents of the human infection mucormycosis that is emerging and continuing to increase in incidence. *Mucor* serves as a model to study light sensing and RNA silencing, *Mucor* produces biodiesel that satisfies American and most European specification standards. In addition, *Mucor* is a dimorphic fungus and its morphogenic transition between yeast and spore/hyphae depends on the concentrations of CO_2 and O_2, which Louis Pasteur discovered more than 100 years ago. Albeit this versatility and unique historical position as Robert Hooke first observed and described *Mucor* spp in detail with his first microscope, the genetics and molecular biology developed for this fungus so far are far from the state of the art for model fungi in the dikarya. Here I present a genetic dissection of the dimorphism of *M. circinelloides* that is orchestrated by calcineurin, a Ca^{2+}/calmodulin-dependent serine/threonine-specific protein phosphatase. I found that in the presence of the calcineurin inhibitor FK506, *Mucor* exhibits multi-budded yeast growth. Mutant analyses revealed that the mutations within the domains where calcineurin and FK506 interact confer resistance to FK506. Disruption of the calcineurin regulatory subunit gene, *cnbR*, results in a complete loss of calcineurin activity and the resulting mutants are locked in permanent yeast phase growth. *M. circinelloides* encodes three calcineurin catalytic A subunits (CnaA, CnaB, and CnaC) and mutants lacking *cnaA*Δ or *cnaB*Δ display different phenotypes, indicating that the two genes are functionally differentiated. Further analyses demonstrate that the morphogenic transition programs different outcomes during host-pathogen interactions. For example, wild-type spores/hyphae display virulence traits during interactions with host immune cells, whereas the yeast-locked mutants lack virulence attribute during their interactions with hosts. These findings advance our understanding of calcineurin as a global fungal virulence factor as calcineurin is required for pathogenesis in human and plan pathogens, including *Candida* spp., *C. neoformans*, *A. fumigatus*, and *M. grisea*..

401. First insights on novel molecular mechanisms involved in the regulation of carotenogenesis in *Fusarium* . Macarena Ruger-Herreros, Obdulia Parra-Rivero, Javier Pardo-Medina, Javier Avalos, Carmen Limon. Genetics, University of Seville, Sevilla, Sevilla, Spain.

The genus *Fusarium* stands out for its complex secondary metabolism. Our group uses the synthesis of carotenoids in *Fusarium oxysporum* and *Fusarium fujikuroi* as a model to investigate novel fungal regulatory mechanisms. The production of carotenoids in these species is stimulated by light through the transcriptional induction of the structural *car* genes. The pathway is repressed through an unknown mechanism by the product of the gene *carS*, coding for a Ring-finger protein with a LON domain. We have identified two types of carotenoid overproducing (COv) mutants: (i) affected in the *carS* gene, and (ii) affected in a non-coding intergenic region (NCIR) upstream to *carS*, identified from the analysis of T-DNA insertional mutants. This communication deals with the molecular mechanism of CarS and the participation of *carS*-linked non-coding RNA, as suggests the bioinformatic identification of two putative miRNA precursors in the NCIR sequence of *F. oxysporum*. The first objective is achieved through the study of CarS protein levels under different regulatory conditions and the search for CarS-interacting proteins. Against our predictions, CarS levels do not correlate inversely with carotenoid biosynthesis. The second objective started with RT-PCR assays, PCR on cDNA samples, and northern blots with NCIR sequences. Our data in *F. oxysporum* show significant expression levels along the NCIR in the wild type, which are affected by illumination and by the occurrence of COv mutations. PCR amplifications on cDNA samples and northern experiments detect non-coding transcripts of less than 1 Kb. The homologous NCIR sequence in *F. fujikuroi* contains also putative miRNA precursors in different relative positions and orientation and exhibits a high degree of sequence divergence. Moreover, similar RT-PCR studies revealed lower levels of non-coding transcripts compared to those of *F. oxysporum*. The genomes of both species contain all the genes for proteins involved in canonical RNA silencing mechanism, as Dicer, Ago and RdRP, supporting the occurrence of small RNA regulatory mechanisms in *Fusarium*.

402. Regulating the regulators: Cleavage Factor I proteins in *Magnaporthe oryzae*. Julio Rodríguez-Romero, Ane Sesma. Centre for Plant Biotechnology and Genomics, Technical University of Madrid, Pozuelo de Alarcon, Madrid, Spain.

Cleavage factor I (CFI) proteins are core components of the polyadenylation machinery that can regulate several steps of mRNA life cycle, including alternative polyadenylation, splicing, export and decay. Rbp35 is a novel protein component of the polyadenylation machinery, and it is present exclusively in filamentous fungi[1]. In *Magnaporthe oryzae*, it regulates alternative polyadenylation of transcripts associated with pathogenicity. Here, we describe the regulatory mechanisms that control the Rbp35/CfI25 complex and Hrp1, another CFI protein. Using mutational, genetic and biochemical studies we demonstrate that cellular concentration of CFI mRNAs is a limited indicator of their protein abundance. Our results suggest that several posttranscriptional mechanisms regulate Rbp35/CfI25 complex and Hrp1 in the rice blast fungus, some of which are also conserved in other ascomycetes[2]. With respect to Rbp35, these include C-terminal processing, RGG-dependent localisation and cleavage, and a C-terminal autoregulatory domain. Our proteomic analyses indicate that Rbp35 also controls cellular levels of protein subsets but it is not required for general splicing or translation.Carbon depletion induces the transcription of two polyadenylated transcripts of ~1,000 (uORF1) and ~750 (uORF2) nucleotides in length that derive from *RBP35* 5'UTR. The uORF1 is required for correct function of the TOR kinase pathway on minimal media. The role of two additional CFI proteins in *M. oryzae*, Hrp1 and CfI25, is further analysed to understand why filamentous fungi have maintained proteins with apparently redundant functions. Our findings uncover broad and multilayer regulatory mechanisms controlling fungal polyadenylation factors, which have profound implications in pre-mRNA maturation.

References

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403. Transcriptomic profiles of the smoke tree wilt fungus *Verticillium dahliae* under nutrient starvation stresses. Dianguang Xiong, Yonglin Wang, Chengming Tian. College of Forestry, Beijing Forestry University, Beijing, China.

Verticillium dahliae is a notorious plant pathogen that causes vascular wilt on more than 200 plant species. There are currently no effective measures to control Verticillium wilt. Since effective pathogen nutrition guarantees successful invasion, understanding fungal nutrient acquisition and nutrient starvation responses are important for developing disease control strategies. Here, we used RNA-Seq to analyze the response of *V. dahliae* to nutrient starvation, including carbon and nitrogen starvation. Gene expression profiles analysis showed that numerous genes were differentially expressed under carbon starvation (852 upregulated genes, 539 downregulated genes) and nitrogen starvation (487 upregulated genes, 291 downregulated genes). Among them, genes involved in utilization or production acetyl-CoA, including glycolysis, fatty acid biosynthesis or metabolism and melanin biosynthesis, were repressed under carbon starvation, while melanin biosynthesis genes were strongly induced under nitrogen starvation. These results, combined with *VDH1* expression data, suggested that melanin biosynthesis and microsclerotia (MS) development were induced under nitrogen starvation, but MS development was suppressed under carbon starvation. Furthermore, many genes encoding carbohydrate active enzymes (CAZys) and secreted proteins were induced under carbon starvation condition. Overall, the results derived from these work should increase our understanding of how *V. dahliae* responds to nutrient starvation and help to identify potential virulence factors for the development of new disease control strategies.

404. *Candida glabrata ADA2* controls thermotolerance and drug tolerance. Shang-Jie Yu, Ying-Lien Chen. Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan.

Candida glabrata, the second most frequent cause of candidiasis after *C. albicans*, is an emerging human fungal pathogen that is commonly drug tolerant, resulting in treatment difficulties and a higher mortality in immunocompromised patients. To search for potential drug targets, we screened a *C. glabrata* mutant library and found an *ada2* mutant susceptible to micafungin. We thus hypothesized that *C. glabrata* Ada2 (a transcriptional Adaptor) controls drug tolerance. To test this hypothesis, we generated two independent *ada2* mutants from the wild-type CBS138. In addition to confirming that *C. glabrata ada2* mutants were susceptible to micafungin, caspofungin and anidulafungin, we found that *ada2* mutants exhibited pleiotropic phenotypes. For example, the *ada2* mutants were susceptible to cell wall-disturbing agents (calcofluor white, congo red and SDS), azoles (fluconazole, voriconazole and posaconazole), and endoplasmic reticulum stress chemicals (tunicamycin and dithiothreitol). Surprisingly, the *ada2* mutants were unable to grow at 40°C, suggesting that Ada2 plays a critical role controlling thermotolerance. According to our RNA sequencing experiments, forty three genes were down regulated in *ada2* mutant compared to the wild type. Two genes associated with thermotolerance phenotype were *HSP30* and *SSB1*, while genes linked with drug tolerance were *ERG6* and *ERG13*. Furthermore, we found that *ada2* mutants were hypersensitive to calcineurin inhibitors FK506 and cyclosporin A, indicating a relationship between Ada2 and the calcineurin signaling cascade. The pleiotropic drug sensitivities rendered by *ADA2* disruption support that Ada2 inhibitors may be synergistic in combination with currently available therapeutic agents for more effective treatment of *C. glabrata* infections.

405. Post-translational regulation of the small RNA biogenesis pathway. Kristina Herbert. Microbiologia, CICESE, Ensenada, Baja California, Mexico.

RNAi pathways are a conserved eukaryotic mechanism, whereby small RNAs direct the posttranscriptional and/or transcriptional regulation of gene expression. Small RNAs have been shown to contribute to transposon silencing, viral defense, DNA elimination, heterochromatin formation, and posttranscriptional repression of cellular genes. Therefore, it is important to determine how small RNA biogenesis is regulated. In metazoans the Microprocessor complex (MC) is responsible for the first step in miRNA biogenesis, cleavage of the pri-miRNA to release a stem-loop structure known as the pre-miRNA, which contains the embedded mature microRNA (miRNA). The MC is made up minimally of Drosha, an RNaseIII-like enzyme, and DGCR8/Pasha, a dsRBD containing protein. The second cleavage step is carried out in the cytoplasm by another RNaseIII enzyme Dicer, which in mammalian cells works together with a dsRBD containing protein, TRBP2. We have already shown in human cells that DGCR8 is a target of the ERK/MAPKs. Expression of phosphomimic

DGCR8 or inhibition of phosphatases increases the levels of expression of DGCR8 and Drosha. The increased levels of phosphomimic DGCR8 are due to an increase in protein stability. MCs incorporating phosphomutant or phosphomimic DGCR8 do not exhibit altered processing activity. However, the increased MC levels, seen for the phosphomimic, lead to enhanced pri-miRNA processing and increased cell proliferation (Herbert Cell Reports 2013). Similarly, the phosphorylation of TRBP2 in human cells by the ERK/MAPKs also leads to increased TRBP2 and DICER protein levels and an upregulation of most miRNAs, except let7, which is downregulated (Paroo Cell 2009). Again this leads to increased growth and proliferation. Although, Drosha and DGCR8 homologs are not found in fungi, minimal RNAi machinery composed of Argonaute and Dicer proteins appear to have been conserved in a majority of the fungal kindom, including filamentous fungi. Given, that both miRNA biogenesis complexes are targets of the MAPK/ERK pathway in human cells, we hypothesize that small RNA biogenesis will be similarly regulated in fungi, and are investigating this in the model Neurospora crassa.

406. Pan-genomic analysis of TamA in *Aspergillus nidulans*: the regulatory network of a dual function Zn(II)2Cys6 transcription factor. Damien Downes[1], Koon Ho Wong[2], Richard Todd[1]. 1) Department of Plant Pathology, Kansas State University, Kansas, USA; 2) Faculty of Health Sciences, University of Macau, Macau SAR, China.

Zn(II)2Cys6 zinc binuclear cluster proteins represent the largest family of transcription factors in fungi and regulate genes of diverse pathways, including primary and secondary metabolism, and development. For most transcription factors the DNA binding domain is considered essential for function. However proteins in the emerging class of dual function transcription factors operate by either direct DNA binding or as protein-binding co-activators and co-repressors (non-DNA-binding factors). The switch between functions can be determined by promoter context and cell type. Dual function has been reported in different cell types for the Hairy repressor in *Drosophilia, Xenopus* and zebrafish as well as mouse SCL/TAL-1. We recently reported the first example of a fungal dual function transcription factor, the *Aspergillus nidulans* Zn(II)2Cys6 transcription factor TamA. TamA is unique in that dual function occurs in the same cell type, where it binds the *gdhA* promoter directly while acting as a co-activator of the global nitrogen GATA transcription factor AreA at *amdS* and *fmdS*. To determine the extent of the TamA dual function regulatory network we have taken a pan-genomic approach. Using FLAG-epitope-tagged TamA for ChIP-seq, we have identified novel targets of TamA, including genes involved in primary metabolism, secondary metabolism and siderophore biosynthesis. This analysis has indicated that TamA target sites lack sequence conformity. Therefore TamA may act via multiple DNA-binding sequences, potentially on the basis of interaction with novel proteins. To determine the TamA activator and co-activator regulons we characterized the effect of TamA on global gene expression using stranded RNA-seq in *tamA*Δ and *tamA*^{C90L} loss-of-DNA binding mutants. The dual functions of transcription factors such as TamA provide an additional level of combinatorial control to mediate gene-specific expression. Analysis of the TamA regulatory network at a pan-genomic level provides insights into how dual function transcription factors facilitate promoter plasticity and act as a vehicle for adaptive evolution.

407. Potential microRNAs regulate the response to injury of the filamentous fungus *Trichoderma atroviride*. Jose Manuel Villalobos-Escobedo, Nohemí Carreras-Villaseñor, Joel Rodríguez-Medina, Cei Abreu-Goodger, Alfredo Herrera-Estrella. LANGEBIO-CINVESTAV, Km 9.6 Libramiento Norte Carretera Irapuato-León. Irapuato, Guanajuato, Mexico.

Wound response in multicellular eukaryotes is essential for survival and is highly conserved in plants and animals. In our laboratory we recently discovered that the filamentous fungus *Trichoderma atroviride* responds to injury by triggering hyphal regeneration and the formation of asexual reproductive structures. Our transcriptomic analysis revealed that the mechanism of response to this stimulus is very similar to that of animals and plants, suggesting that it is highly conserved amongst the three eukaryotic kingdoms. Additionally, several recent studies have reported that post-transcriptional regulation by microRNAs is involved in the response to injury in animals and plants.

Based on this background we decided to evaluate the injury response in mutants of the RNAi synthesis machinery of *T. atroviride*. The *Δdcr2* and *Δrdr3* strains presented a dramatic defect in regeneration ability and asexual reproduction in response to injury. To understand the molecular processes affected by the absence of the RNAi pathway, we performed transcriptomic analysis of the WT and *Δdcr2* strains subjected to injury, showing that signaling processes, DNA repair and cell cycle progression are essential to overcome this stress and are affected in the *Δdcr2* mutant. Even more interesting was the presence of a population of small RNAs of 21-22nt in response to injury in the WT, which is absent in Δdcr2 mutant, implicating them as a product of the Dcr2 enzyme. microRNA prediction allowed to determine that most 21-22 nt small RNAs correspond to microRNAs.

Our results indicate that gene regulation by putative-microRNAs is essential to respond to injury in *T. atroviride.* This phenomenon opens the possibility of demonstrating the relevance of post-transcriptional regulation by microRNAs in filamentous fungi in response to environmental stimuli. Furthermore, the knowledge generated may be helpful in understanding the fine regulation by microRNAs of the tissue repair process, which could culminate in enhancing regenerative therapies in humans.

408. Novel function of Swi3 in moderating aerobic respiration and oxygen consumption in *Saccharomyces cerevisiae*. Sneha Lal, Jagmohan Hooda, Md Maksudal Alam, Ajit Shah, Thai Cao, Zhenyu Xuan, Li Zhang. Molecular and Cell Biology, University of Texas at Dallas, Richardson, TX.

Aerobic cellular respiration is vital for production of energy in eukaryotes ranging from yeast to humans. Previous studies in our lab with *Saccharomyces cerevisiae* have shown that many target genes of SWI/SNF components are oxygen-regulated, and hence may play an important role in the regulation of aerobic respiration and cellular energy production. Preliminary work in our lab indicates that Swi3, a component of SWI/SNF complex, is a novel regulator that moderates aerobic respiration and oxygen metabolism. Fluorescent live cell imaging in our lab showed that oxygen is required for the nuclear localization of Swi3, but not for Swi2. We see a significant increase in the rate of oxygen consumption in *Δswi3* cells, whereas *Δswi2* cells show a similar rate of oxygen consumption as the parent cells. Analysis

of the mitochondrial respiratory chain complex proteins by Blue Native polyacrylamide gel electrophoresis shows higher levels of expression in *Δswi3* as compared to *Δswi2* and the parent cells. Moreover, the promoter activities of two aerobic respiration genes, CYC1 and CYC7, were dramatically increased by *Δswi3* only in heme-sufficient cells. The human homologs of Swi3, BAF155 and BAF170 are known tumor suppressors. Genome-wide ChIP-Seq data in HeLa cells shows that BAF155 and BAF170, but not Brg1 (Swi2 homolog), are associated with many genes encoding functions required for oxidative phosphorylation. Further, we see increased rate of oxygen consumption in HeLa cells when BAF155 or BAF170 is knocked down. Collectively, these results support the idea that Swi3 has a novel function in moderating aerobic respiration gene expression and oxygen metabolism. We are further investigating the mechanism of oxygen sensing by Swi3 and its human homologs.

409. Transcriptome analysis of *Aspergillus flavus* reveals *veA*-dependent regulation of secondary metabolite gene clusters, including the novel aflavarin cluster. J.W. Cary[1], P. Harris-Coward[1], Y. Yi[2], J.M. Lohmar[2], S. Shantappa[2], M. Johns[2], J. Yu[1], D. Sorensen[2], H. Shen[2], H. Zheng[3], S. De Saeger[3], J. Diana. Di Mavungu[3], A.M. Calvo-Byrd[2]. 1) Food and Feed Safety Research Unit, USDA/ARS, Southern Regional Research Center, New Orleans, LA 70124, USA; 2) Department of Biological Sciences, Northern Illinois University, 155 Castle Drive,DeKalb, IL 60115,USA; 3) Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium.

The global regulatory gene, *veA*, governs development and secondary metabolism in numerous fungal species, including *Aspergillus flavus*. This is especially relevant since *A. flavus* infects crops of agricultural importance worldwide, contaminating them with potent mycotoxins. The most well known are aflatoxins, toxic and carcinogenic polyketide compounds. The expression of the aflatoxin gene cluster is dependent on the global regulator *veA*. Studies of the *A. flavus* genome revealed 56 gene clusters possibly connected to the synthesis of secondary metabolites. Many of these metabolites are still unknown or the association between a known metabolite with a particular gene cluster has not yet been established. In the present transcriptome study we show that *veA* is necessary for the expression of a larger number of genes. The expression of several uncharacterized secondary metabolite gene clusters is also *veA*-dependent. One of these clusters under the influence of *veA* is cluster #39. Absence of *veA* results in a down-regulation of the five genes found within this cluster. Interestingly, our results indicate that the cluster is mainly expressed in sclerotia. Chemical analysis of sclerotial extracts revealed that cluster #39 is responsible for the production of aflavarin, a compound previously isolated from sclerotia of *A. flavus* that possesses insecticidal activity.

410. Functional characterization of the long non-coding RNA *RZE1* in *Cryptococcus neoformans*. Nadia Chacko[1], Youbao Zhao[1], Ence Yang[2], Linqi Wang[1], James Cai[2], Xiaorong Lin[1]. 1) Dept Biol, Texas A&M Univeristy, College Station, TX; 2) Vet School, Texas A&M Univeristy, College Station, TX.

Like many other fungal pathogens, pathogenicity and morphogenesis are tightly associated in *Cryptococcus*. Previously, we established that a master regulator Znf2 bridges cryptococcal morphotype and virulence potential. Activation of Znf2 promotes filamentous growth, elicits host defense responses, and protects hosts against subsequent lethal infections. Thus, knowledge of the regulation of Znf2 could be exploited to design measures to alleviate cryptococcosis. By screening random insertional mutants for *znf2*Δ-like phenotypes, we identified a novel regulator of Znf2, *RZE1*. Deletion of *RZE1* inactivated the *ZNF2* regulon and locked cells in the yeast form. Interestingly, the disruption of *RZE1* exerted only modest effect on the expression level of *ZNF2* and the *ZNF2* transcript isoform appears to be the same in the wild type and the *rze1*Δ mutant. Targeted site mutagenesis indicates that *RZE1* functions as a transcript, not as proteins. Thus, *RZE1* is the first bona fide lncRNA functionally identified in human fungal pathogens. Heterokaryon assays demonstrate that *RZE1* is functionally restricted to its native nuclei. Elucidation of *RZE1*'s mode of action would provide a paradigm for future research on lncRNAs in fungal pathogens.

411. Histidine kinase pathway components are required for growth in the parasitic form of *Histoplasma capsulatum*. Sinem Beyhan[1], Giselle Knudsen[1], Anita Sil[1,2]. 1) Microbiology and Immunology, Univ California, San Francisco, San Francisco, CA; 2) Howard Hughes Medical Institute.

Histoplasma capsulatum is a respiratory fungal pathogen of humans and has a dimorphic life cycle, switching from an infectious filamentous form in the soil to a pathogenic yeast form in mammalian hosts. In the laboratory, *H. capsulatum* grows in the yeast form at 37°C and in the filamentous form at room temperature. We previously identified four transcription factors, Ryp1-4, and showed that they are key regulators of a temperature-responsive genetic network that is required for yeast-phase growth in *H. capsulatum*. In this study, we performed immunoprecipitations using antibodies against Ryp2 and Ryp3, followed by mass spectrometry to identify Ryp2- and Ryp3-interacting proteins at 37°C. Among the diverse set of proteins that interact with Ryp2 and Ryp3, we characterized two Ryp2-interacting proteins with predicted response regulator domains. Response regulators and sensor histidine kinases form two-component regulatory systems that are often involved in sensing environmental signals using a phosphorelay mechanism. Because it was previously shown that a sensor histidine kinase, Drk1, is required for the yeast-phase growth (Nemecek et al 2006 *Science*), we hypothesized that the two Ryp2-interacting response regulators could also be important for yeast-phase growth in *H. capsulatum*. Confirming our hypothesis, knockdown of these two genes (named *RYP5* and *RYP6*) resulted in filamentous growth regardless of temperature, indicating that they are required for yeast-phase growth. Additionally, from our previous studies, we found that *RYP5* and *RYP6* are not regulated (directly or indirectly) by Ryp1-4, suggesting that Ryp5 and Ryp6 may act upstream of Ryp1-4. We are currently investigating whether Ryp5 and Ryp6 act together with Drk1 and upstream of Ryp1-4 to regulate yeast phase growth. These experiments are highly significant since they will reveal the importance of two-component regulatory systems for sensing host temperature and provide a molecular understanding of how a pathogenic fungus responds to host temperature to cause disease.

412. Role of MAP kinase pathways in the pathogenicity of the wheat pathogen *Zymoseptoria tritici*. M.-H. Lebrun[1], E. Marchegiani[1], J. Vallet[1], S. Deller[2]. 1) INRA 1290 BIOGER, INRA, Thiverval-Grignon, France; 2) Syngenta Limited, European Regional Centre, GU2 7YH, United Kingdom.

Mitogen-activated protein kinases (MAPKs) are essential components of fungal signaling pathways involved in developmental processes, response to stresses and host infection. *Zymoseptoria tritici*, the causal agent of *Septoria tritici* leaf blotch (STB) of wheat, has three MAPK pathways that are all required for infection (ZtFus3 , ZtHog1, ZtSlt2; Cousin et al., 2006; Mehrabi et al., 2006a, Mehrabi et al., 2006b). We showed that *Ztfus3* and *Zthog1* null mutants are non-pathogenic on intact wheat leaves, but reduced in pathogenicity when infiltrated into leaf tissues by syringe injection (reduced necrosis, low number of pycnidia). This rescue by injection suggests that their are mainly unable to penetrate into host leaves. *Ztslt2* null mutant is non-pathogenic both on intact wheat leaves and after injection. This mutant has also a dramatic developmental defect, as *Ztslt2* null mutant older colonies are not hydrophobic, nor melanized, and do not differentiate aerial hyphae and biofilms. All these features are characteristic of aging *Z. tritici* colonies. This switch occurs faster on PDA at 25°C (5 dpi) compared to 18°C (7 dpi) or to YPD (18 dpi). In the most favorable condition (PDA, 25°C, at least 3 dpi), ZtSlt2 is required for the expression of most genes involved in DHN melanin biosynthesis, and genes encoding hydrophobins and alpha-1,3-glucan synthases. Therefore, ZtSlt2 is essential for controlling gene expression during this developmental switch. We have explored this phenomenon and compared whole genome expression (RNAseq) between wild type and *Ztslt2* null mutant.

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Mehrabi et al. (2006a), Molecular Plant-Microbe Interactions 19(4): 389-398.
Mehrabi et al. (2006b), Molecular Plant-Microbe Interactions 19(11): 1262-1269.

413. Cyclin Transcription is Impacted by Nuclear Positioning in *Ashbya gossypii*. Samantha Roberts[1], Abhishek Kumar[2], Hari Shroff[2], Amy Gladfelter[1]. 1) Biological Sciences, Dartmouth College, Hanover, NH; 2) 2Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, US NIH, Bethesda, MD.

In multinucleate cells of *Ashbya gossypii*, nuclei divide independently despite sharing a common cytoplasm. Internuclear distance is tightly regulated, with neighboring nuclei ~5 μm apart. We demonstrated that heterogeneously localized cyclin transcripts associated with protein aggregates promotes asynchronous division. To determine whether variability in transcript production also promotes transcript spatial heterogeneity, nuclei producing cyclin transcripts were identified using single molecule RNA FISH. Cyclin transcription correlated with the cell cycle phase of the nucleus indicating that even in a shared cytoplasm nuclei have "knowledge" of their cell cycle phase. It is possible that variable transcription of cyclins contributes to variable local cytosolic concentrations of cyclin protein. If so, the volume of cytosol between neighboring nuclei may be crucial to nuclear cycle autonomy. In fact, previous data showed when nuclei cannot control their spacing they undergo more synchronous division. To further investigate the relationship between nuclear spacing and division autonomy we utilized a mutant strain in which large nuclear clusters form with 10-30 nuclei <0.5 μm apart from each other. Surprisingly, we found that asynchrony, nuclear cycle stage profiles, and the variability in cyclin transcription in clusters of nuclei are comparable to wild type. Interestingly, all genes examined were transcribed by a higher proportion of nuclei in clusters except G1 cyclins. However, the G1 cyclins were transcribed by a higher proportion of mitotic nuclei than in the wild type. These data suggest a global transcriptional increase, consistent with sharing of a transcription factor. We have data to suggest that the transcription factor Swi4 contains an intrinsically disordered domain and heterogeneously localizes within *Ashbya* cells. These results suggest that protein aggregation to promote heterogeneous localization is a mechanism used in multiple cellular processes to create functional regions within the continuous cytoplasm of large filamentous cells.

414. Systematic deletion of homeobox genes in *Aspergillus flavus* reveals their roles in development. Jeffrey Cary, Pamela Harris-Coward, Brian Mack, Les Scharfenstein, Perng Kuang Chang. US Department of Agriculture, ARS-SRRC, New Orleans, LA.

Homeobox proteins are a class of transcription factors that are well conserved in many eukaryotes including fungi. The homeodomain (HD) typically consists of about 60 amino acids and is capable of recognizing and binding to specific DNA sequences through the recognition property of the homeodomain. Therefore, homeo proteins are believed to regulate the expression of targeted genes, especially those involved in development. Studies in several filamentous fungi have shown that homeobox genes are required for normal conidiogenesis and fruiting body formation. To determine the role of homeobox genes in the aflatoxin-producing ascomycete, *Aspergillus flavus*, we generated knockout mutants of eight putative homeobox genes and analyzed their functions in production of conidia, sclerotia, and aflatoxin. In general, conidial production in the mutants was higher than that observed in the control with the exception of the AFLA_026100 and AFLA_ 069100 mutants where production was significantly lower and absent, respectively. In addition, sclerotial production was lower in all mutants and completely absent in the AFLA_ 069100 mutant. Production of aflatoxin was not significantly different in the mutants compared to that of the control with the exception of the AFLA_ 069100 mutant that failed to produce detectable levels. These data suggest that the majority of the homeobox genes analyzed negatively regulate conidiation and positively regulate sclerotial formation. AFLA_069100, the ortholog of the *Magnaporthe oryzae hox2* and *Fusarium graminearum htf1* homeobox genes, appears to serve as a positive regulator of conidial and sclerotial development as well as aflatoxin production in *A. flavus*. This homeobox gene should be of interest as a target for strategies to control aflatoxin contamination of susceptible food and feed crops.

415. Transcription factor Zfp1 controls effectors required for full pathogenesis in *Ustilago maydis*. Kitty Cheung[1], Michael Donaldson[1,2], Kelsey Spence[3], Barry Saville[1,2]. 1) Environmental and Life Sciences, Trent University, 2140 East Bank Dr., Peterborough, Ontario, Canada; 2) Forensic Science Program, Trent University, 2140 East Bank Dr., Peterborough, ON, Canada; 3) Department of Population Medicine, Ontario Veterinary College, University of Guelph, 50 Stone Road E., Guelph, ON, N1G 2W1, Canada.

The plant pathogen *Ustilago maydis* must establish a biotrophic relationship with the host *Zea mays* to complete its life cycle. To facilitate this process, *U. maydis* produces effectors: secreted proteins that alter the host to favour fungal growth. The functions of effectors have been investigated by many researchers; yet the control of their expression is largely unknown. We have identified a *U. maydis*

transcription factor, zinc finger protein 1 (Zfp1), that, when deleted, changes the transcript levels of confirmed and predicted effector encoding genes. Similar to previously characterized zinc cluster transcription factors, Zfp1 contains a GAL4-like Zn(II)2Cys6 binuclear cluster DNA-binding domain and a fungal specific transcription factor domain. To investigate the involvement of these domains in protein function, *U. maydis* strains were created in which the domains were mutated or deleted. Subcellular localization was determined using Zfp1-eGFP fusion protein constructs. Deletion of *zfp1* resulted in a reduction in anthocyanin and the arrest of pathogenic development at the leaf tumour stage. Microscopic investigation showed fungal development is attenuated and remains mostly in the leaf tissue, supporting macroscopic observations. Complementation with wildtype *zfp1* restores pathogenesis and anthocyanin production. Putative Zfp1 target genes were identified by comparative RNA-seq analysis and confirmed by qPCR. Of the genes with a significant difference in transcript levels, 111 encode predicted effectors. One of the characterized effectors identified is Tin2, which when deleted had a similar phenotype to that of the *zfp1* deletion strains. This is consistent with Zfp1 influencing the transcription of *tin2* during *in planta* growth. Our findings indicate that Zfp1 contributes to *U. maydis* pathogenic development by directly or indirectly regulating the expression of effector genes.

416. Potential protein interactions of AreB, the nitrogen regulator in *Aspergillus nidulans*. Damian Garbicz[1], Maria Macios[1], Dominik Cysewski[2], Piotr Weglenski[3], Agnieszka Dzikowska[1,2]. 1) Institue of Genetics and Biotechnology, University of Warsaw , Warsaw , Poland; 2) Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; 3) Centre of New Tecnnologies, University of Warsaw, Poland.

Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In *Aspergillus nidulans* this regulatory mechanism depends on GATA transcription factors AreA and AreB. Activity of these factors is also regulated by carbon regime. areB gene encodes three different proteins which differ at their N-terminal part. To explore the mechanism of transcription regulation by AreB, we identified proteins which interact with this regulator in vivo. Potential protein partners of AreB were identified using the strain expressing AreB-β::TSapphire fusion and immunoprecipitation with GFP-Trap®_A (Chromotek). Proteins were identified using Mass Spectrometry (MS) analysis. Several proteins interacting with AreB-β under different carbon / nitrogen conditions were identified, like different transcription factors, protein kinases / phosphatases and importines.

417. Regulation of the *N*-acetylglucosamine catabolism gene cluster by an NDT80-like transcription factor in filamentous fungi. L. Kappel, R. Gaderer, V. Seidl-Seiboth. Vienna University of Technology, Vienna, Austria.

The abundant amino sugar *N*-acetylglucosamine (GlcNAc) is a key constituent of chitin and peptidoglycan. Chitin, the second most abundant biopolymer with a natural turnover of at least 10^9 tons per year, stars not only as structural component but also as nutrient source for microorganisms. Chitinolytic enzymes have already been subject to a number of studies, particularly in *Trichoderma* spp. Metabolism of GlcNAc, though, has so far only been investigated in bacteria and the human pathogenic yeast *Candida albicans* where the focus rather was laid on its role in virulence and cell signaling. Genome analysis showed that in filamentous fungi the genes encoding the GlcNAc catabolism enzymes are clustered and in addition the cluster contains an NDT80-like transcription factor. In yeast *Saccharomyces cerevisiae* and also in filamentous fungi, e.g. *Neurospora crassa*, these NDT80-like transcription factors are involved mainly in reproductive events. In *C. albicans* the transcriptional regulator of the GlcNAc gene cluster is not known. GlcNAc is an easily assimilable carbon source for *Trichoderma* spp. In *T. reesei* and *T. atroviride* the expression levels of the catabolic genes were increased strongly upon growth on GlcNAc, whereas the transcription factor was only weakly upregulated. Deletion of any of the cluster genes in *T. reesei* resulted in a severe growth defect on GlcNAc, which shows that each of these enzymes is unique for the respective function in GlcNAc catabolism. We could show that in *T. reesei* the GlcNAc genes are positively regulated by the NDT80-transcription factor, which we termed RON1 (Regulation of N-acetylglucosamine catabolism). Deletion of *ron1* resulted in loss of expression of all the GlcNAc cluster genes. Thus, with RON1 we identified the main regulator of GlcNAc catabolism in fungi. Interestingly, a double knockout of the first and third catabolic enzyme resulted in generation of pale conidia, which was not observed for single knockout strains or any other combination of double- or the triple mutants in this cluster. This finding hints at a second role of GlcNAc catabolism genes in vegetative development.

418. Molecular mechanism underlying the heme regulation of Gis1 activities. Purna Chaitanya Konduri, Ajit Shah, Michael Comer, Sneha Lal, Li Zhang. Molecular and Cell Biology, University of Texas at Dallas, Richardson, TX.

The ability to sense environmental cues and respond to them appropriately is critical for the existence of any living cell. In yeast, Gis1 is a DNA-binding transcription factor that induces the transcription of stress response genes during post diauxic shift. Gis1 also senses changes in oxygen and heme levels. Hence, yeast Gis1 is a unique transcriptional regulator. It belongs to JHDM3/JMJD2 subfamily of demethylases and is highly homologous to the mammalian JmjC domain-containing JMJD2B protein. JMJD2B plays an important role in histone demethylation, oxygen regulation and hormonal signaling. The JmjC domain forms an enzymatically active pocket that coordinates Fe(III) and alphaKG. Purification of Gis1 followed by biochemical assays indicates that heme directly binds Gis1. The aim of this research is to understand the mechanism by which Gis1 responds to changes in heme levels. Various domain deletions of Gis1 indicate that there may be more than one site important for heme binding, bringing about its regulation. DNA binding properties of Gis1 were also studied for these constructs in the presence and absence of heme. Domain interaction and DNA binding studies will offer a comprehensive molecular understanding of how Gis1 senses heme levels and promotes gene regulation.

419. Effector gene regulation in the fungus *Parastagonospora nodorum*. Kar-Chun Tan[1], Kasia Rybak[1], Huyen Phan[1], Peter Solomon[2], Richard Oliver[1]. 1) Environment & Agriculture, Curtin University, Perth, Australia; 2) Plant Sciences Division Research School of Biology, The Australian National University, Canberra 0200, Australia.

Parastagonospora nodorum is the causal agent of septoria nodorum blotch on wheat. The fungus produces a series of necrotrophic effectors to facilitate infection on hosts that carry dominant sensitivity/susceptibility genes. Known *P. nodorum* effector genes include *ToxA*, *Tox1* and *Tox3*. Current experimental evidence suggests that there are many more undiscovered effector genes. In a mutagenesis

study, we have recently deleted a gene that encodes a putative GAL4-like transcription factor. Mutants without the putative transcription factor gene remained fully pathogenic on wheat varieties that carry dominant Tox1 and Tox3 sensitivity genes. However, preliminary studies indicate the putative transcription factor gene knockout mutants were unable to infect wheat with the *Tsn1* genotype (ToxA sensitive). Furthermore, ToxA expression is down-regulated in the knockout mutant. We anticipate that the putative transcription factor may function to regulate the expression of novel effector genes. As such, experiments currently underway to transcriptionally profile these mutants.

420. A novel transcription factor plays a critical role in the inhibition of glycoside hydrolase family 11 in *Trichoderma reesei*. R. Liu, L. Chen, Y. Jiang, Z. Zhou, G. Zou. Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences,Shanghai 200032, China.

In previous studies, all the identified transcription factors take the similar effects on the expression level of cellualse genes and hemicellulose genes in the hyper-producing species, *Trichoderma reesei*. In this study, we screened a novel transcription factor SxlR, which specially regulated the transcription of genes coding glycoside hydrolase family 11 (GH11) xynlases. The deletion of SxlR in *T. reesei* Rut-C30 (mutant Δ*sxlR*Rut-C30) resulted in the 150% increase of xylanase activity in inducing medium. By contrary, over-expression of SxlR brought 15% decrease. No significant difference in cellulase activity and extracellular protein among Rut-C30, Δ*sxlR*Rut-C30 and the overexpression strain. The above results indicated SxlR might be a negative regulator for hemicellulase. Further analysis of the expression level of potential xynlase genes demonstrated that only genes coding glycoside hydrolase family 11 (GH11) xynlases (*xyn1, xyn2, xyn5*) were observed significant changes in SxlR deletion/over-expression mutants. Electrophoretic mobility shift assay (EMSA) showed obvious gel shifts were detected for SxlR binding domain with all the promoters of GH11 xynlase while no gel shifts for the SxlR binding domain with the promotors of other xylanase genes. It indicates SxlR plays a critical role in the inhibition of glycoside hydrolase family 11 in *Trichoderma reesei*.

421. Transcriptome analysis of nonself recognition-associated programmed cell death in the chestnut blight fungus, *Cryphonectria parasitica*.. Anatoly Belov, Myron Smith. Department of Biology, Carleton University, Ottawa, ON, Canada.
Anatoly Belov[1] and Myron L. Smith[1] 1.Department of Biology, Carleton University, Ottawa, Ontario, Canada.

In this study we use the tree pathogen, *C. parasitica*, and a dsRNA virus that infects this fungus, to understand mechanisms of fungal nonself recognition-associated Programed Cell Death (PCD). PCD can result when incompatible fungal strains fuse during vegetative growth, and thus serves as a barrier to the horizontal transmission of disease elements, including viruses. To seemingly combat this defense mechanism, expression of the CHV1 virus-encoded p29 protein in *C. parasitica* results in a delay in nonself recognition-associated PCD, and thus enhanced virus transmission. Transcriptome analysis by next-generation sequencing of cDNA from mycelia with cells undergoing *vic3*-associated PCD revealed that 146 genes are differentially expressed compared to compatible fusion controls (p-value < 0.001). The expression of CHV1-p29 in one of the paired incompatible strains resulted in significantly altered transcript levels of 188 genes compared to control incompatible interactions in which the viral element was absent; this may provide some leads into how the virus interferes with nonself recognition-associated PCD. Interestingly, the data indicate that incompatible hyphal fusions, but not compatible fusions, activate genes involved in the sexual cycle. Both transcriptome and qPCR analyses showed that transcript abundance of mating-type genes (*MAT1* and *MAT2*) increases by 10-50 fold gradually over time during growth, whereas transcript abundance of the sex pheromone genes (mf1-1 and mf2-1) increases dramatically by more than 1000-fold in cells undergoing *vic3*-associated PCD. Gene enrichment analyses of cells undergoing nonself recognition-associated PCD corroborate the involvement of sex pheromone genes and additionally demonstrate activation of genes generally associated with oxidative processes and apoptosis, and asexual sporulation in fungi.

422. Regulation of *Histoplasma* Cell Shape and Virulence by Temperature. Sinem Beyhan[2], Sarah Gilmore[1,2], Mark Voorhies[2], Anita Sil[1,2]. 1) Howard Hughes Medical Institute; 2) Dept Microbiol/Immunology, UCSF, San Francisco, CA.

The long-term goal of our research is to determine how environmental signals such as temperature regulate morphology and virulence in the fungal pathogen *Histoplasma capsulatum. H. capsulatum* grows in a filamentous form in the soil; once inhaled into a mammalian host, these cells switch their growth program to a parasitic yeast form that subverts the innate immune system to cause disease. Temperature is a key signal that regulates this morphogenetic switch. We identified four transcriptional regulators required for growth in the yeast form in response to host temperature. These factors, named Ryp1, Ryp2, Ryp3, and Ryp4, are homologous to key developmental regulators in other fungi, and represent critical elements of the temperature-dependent regulatory circuit in *H. capsulatum*. Here we explore regulation of Ryp factors and use RNAseq and ribosomal profiling to define genome-wide transcriptional and translational regulation in response to temperature shifts. This work defines molecular paradigms that promote cellular differentiation of this fungal pathogen in response to environmental signals.

423. Rapamycin increases accumulation of cell death regulator VIB-1 in the filamentous fungus *Cryphonectria parasitica*. D. Ren, D Price, I. Hansen, A. Dawe. Biology, New Mexico State University, Las Cruces, NM.88003.

Genetic regulation of vegetative incompatibility, (VI) has been hypothesized to be a means of limiting the spread of mycoviruses. Compatible strains will form a stable heterokaryon, while incompatible strains will seal fused compartments that subsequently undergo programmed cell death. In *Neurospora crassa,* VIB-1 is required for the expression of downstream effectors leading to VI. We have explored the role of a putative *vib-1* homolog from the plant pathogen *Cryphonectria parasitica*, a model system for mycovirus-host interactions. The *Δvib-1* mutant showed enhanced pigmentation and conidiation, and a failure in the VI response between strains that contained allelic variations at loci that control incompatibility. Illumina-based trancriptome profiling was used to compare the genes altered in expression between the wild type strain and the *Δvib-1* mutant, mapping the data to the predicted open reading frames derived from the complete genome sequence. Preliminary analysis has indicated that 878 transcripts were altered, 80 % of which were down-regulated. In

order to begin to identify direct downstream factors regulated by VIB-1, we have used a FLAG-tagged VIB-1 to monitor protein accumulation in response to Rapamycin, a macrolide with antifungal activity that has been used to mimic VI-induced cell death. Consistent with observations in *N. crassa* that have established a role for VIB-1 in starvation responses, under carbon and nitrogen limiting conditions the inhibition of vegetative growth by rapamycin was reduced in the *Δvib-1* mutant. Similarly, the reduction of viability, as measured by the tetrazolium-based MTT assay, was reduced for the *Δvib-1* strain in comparison to wild type when both were exposed to inhibitory concentrations of rapamycin. Using a constitutive promoter to drive expression of the FLAG-tagged construct, we have also observed increased accumulation of VIB-1 protein independent of transcript level in response to rapamycin. Our results suggest that regulation of VIB-1 occurs post-transcriptionally, and also provide a platform for identification of downstream VIB-1 targets.

424. Genetic analysis of multi-drug-resistance (MDR) in *Mycosphaerella graminicola (Zymoseptoria tritici)* field isolates. Selim Omrane[1], Colette Audéon[1], Amandine Ignace[1], Clémentine Duplaix[1], Hind Sghyer[1], Lamia Aouini[2], Gert Kema[2], Anne-Sophie Walker[2], Sabine Fillinger[2]. 1) INRA, AgroParisTech, UMR BIOGER, Avenue Lucien Brétignières, F78850 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*, but also plant pathogenic fungi, e.g., *Botrytis cinerea*, *Oculimacula yallundae* and *Mycosphaerella graminicola* are concerned by this phenomenon.

MDR strains were detected in septoria leaf blotch (*M. graminicola*) field populations since 2008. These strains are cross-resistant to fungicides with different modes of action due to active fungicide efflux. In a previous study, we identified the *MgMFS1* gene overexpressed in all tested MDR field strains (Omrane et al., 2015). This gene encodes a major facilitator membrane transporter whose inactivation abolished the MDR phenotype in at least one field strain.

We went out to identify the mutation(s) responsible for MDR phenotype in two isolated strains (MDR6 and MDR7). Crosses between both MDR strains showed that *mdr6* and *mdr7 loci* are closely linked. A bulk-segregant analysis coupled to next generation sequencing showed a clear co-segregation between phenotypes and the left arm of chromosome 7. This region harbors 14 genes including the gene. We identified a 519 bp insert (LTR-like) in both MDR strains as well as in other (but not all) MDR field strains. Genotyping of the progenies for the promoter insert showed a clear, but not exclusive correlation between the *MgMFS1* promoter insert and the MDR phenotype. These results indicate that the LTR-like insert is responsible for the MDR phenotype, potentially *via MgMFS1* overexpression, but also that an additional and independent mutation confers the MDR phenotype to strain MDR6.

425. Mutation and role of white collar homologues genes in life-cycle of *Ustilago maydis*. M.O. Camargo Escalante, J.A. Sánchez Arreguín, C. G. León Ramirez, J. L. Cabrera Ponce, J. Ruíz Herrera. Genetic Engineering, Cinvestav-Irapuato, Guanajuato, Irapuato, Mexico.

The genome of *Ustilago maydis* contains genes that are homologues to *White Collar* (*WC*) genes of *Neurospora crassa* that we have denominated *umwc-1a*, *umwc-2* and *umwc-1b*. Since it has not been described any light response of this fungus, we proceeded to their mutation by double-joint technique to determine their role. It has been described that *Ustilaginomycetes* do not develop basidiocarps. Nevertheless we recently found that *Ustilago maydis* under experimental conditions they can form basidiocarps. In our observation we noticed that *wc-2* and *wc-1b* mutants are not able to produce basidiocarps but *wc-1a* does. This suggests that a photoreceptor complex might be involved in controlling development of fruiting bodies. Previously it has also been shown in several other organisms that blue light generates changes in carbon metabolism; hence we measured the growth in minimal medium with glucose. We observed that the growth rate of wild-type strain increased in the presence of light compared to darkness, but growth rate of *wc-1a and wc-2 mutants*, except *wc-1b*, were more increased in both condition. Since ROS (Reactive Oxygen Species) response is triggered by stress conditions in plants, such as pathogen invasion, we evaluated the sensitivity of *wc* mutants to H_2O_2. We noticed that the *wc-1a* and *wc-2* mutants are more sensitivity than the wild-type strain, but *wc-1b* mutant had the same behavior as wild type. We conclude that *wc* genes in *U. maydis* regulate positively the process of basidiocarps formation and metabolism of ROS but negatively the growth in glucose.

426. Programmed stop codon readthrough leads to dually targeted protein isoforms. Alina C. Stiebler[1], Johannes Freitag[1,3], Kay O. Schink[2], Thorsten Stehlik[1,4], Julia Ast[1], Britta A. M. Tillmann[1], Michael Bölker[1,4]. 1) Biology, Philipps University, Marburg, Germany; 2) Faculty of Medicine, Centre for Cancer Biomedicine, University of Oslo, Montebello, Oslo, Norway; 3) LOEWE Excellence Cluster for Integrative Fungal Research (IPF), Senckenberg Society, Frankfurt am Main, Germany; 4) LOEWE Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany.

Translation of mRNA into protein is generally a very accurate process. However, programmed translational recoding is widely used in viruses to expand the coding capacity of their genomes. We have recently shown that in a wide range of fungal species programmed translational readthrough of stop codons is used to generate C-terminally extended isoforms of glycolytic enzymes. The extended isoforms carry a C-terminal signal sequence (PTS1) that mediates targeting to peroxisomes.

We characterized the sequence requirements for efficient translational readthrough and identified a short conserved stop codon context (UGA CUA). Genomic screening revealed that this motif occurs in a number of genes that encode important metabolic enzymes both in fungi and animals. We could show that ribosomal readthrough of these genes also results in the formation of peroxisomal isoforms. Overall, our data indicates that programmed stop codon readthrough is a common mechanism to reach a dual localization of enzymatic activity both in the cytosol and in peroxisomes in particular of enzymes implicated in redox homeostasis.

427. Coordinate Regulation by Ammonium Transporters and the *b* locus of *Ustilago maydis* of Genes Involved in Mating and Pathogenicity. Margaret Wallen[1], Jinny Paul[1], Chen Zhao[2], Tieliu Shi[2], Michael Perlin[1]. 1) Biology, Program on Disease Evolution, University of Louisville, Louisville, KY; 2) Bioinformation Center, Key Laboratory of System Biology and Shanghai Information Center for Life Science, Shanghai Institutes for Biological Sciences, The Chinese Academy of Sciences, Shanghai 200032, China.

The dimorphic switch from budding to filamentous growth form is an essential morphogenetic transition that many fungi utilize to cause disease in the host. Although different environmental signals can induce filamentous growth, the developmental programs associated with transmitting these different signals may be the same at one or more levels. In the present study, we explore the relationship between filamentation and expression levels of ammonium transporters (AMTs) that also sense low ammonium for *Ustilago maydis*, the pathogen of maize. Filamentous growth induced in response to low ammonium availability has not heretofore been shown to be essential for pathogenicity. Overexpression of the high affinity ammonium transporter, Ump2, under normally non-inducing conditions, results in filamentous growth. Further examination of AMT expression levels and their influence on signal transduction pathway genes revealed that *ump2* expression levels are correlated with the expression of genes involved in the mating response pathway and in pathogenicity; these genes were further affected by expression levels of Ump1, the low affinity ammonium transporter. Ump1 and Ump2 also affect transcript levels of genes expressed during either filamentous growth or during growth of the fungus inside the host. Finally, deletion of both ammonium transporters seriously impairs disease progression in the host.

Pathogenic and Mutalistic Interactions

428. Application of next generation sequencing in identifying T-DNA insertion loci in a fungal mutagenesis population. Liangsheng Xu[1], Weidong Chen[1,2]. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Washington State University, Pullman, WA.

Agrobacterium tumefaciens-mediated transformation has been widely used in generating random and tagged mutations in fungi. After identifying desired phenotypic changes, it is necessary to identify the T-DNA insertion loci in order to characterize the disrupted genes. A number of techniques, such as TAIL-PCR, inverse PCR and plasmid rescue, have been developed to identify T-DNA insertion loci. In some cases, however, T-DNA insertion loci could not be readily identified using these traditional methods. During our screening of about 800 T-DNA transformants of the plant pathogenic fungus *Sclerotinia sclerotiorum*, a dozen or so transformants showed reduced virulence. Using inverse PCR or plasmid rescue methods, we were able to successfully determine the flanking sequences of the T-DNA insertion in only four of the transformants. The T-DNA insertion loci in the remaining nine transformants could not be located using the traditional methods. Therefore, we applied next-generation to sequence the whole genomes of the transformants. Genomic DNA libraries of the transformants were barcoded, pooled and subjected to sequencing using Ion Proton system. We obtained a total of about 89.8 million reads with average length of 136 bps. After separating the reads based on barcodes, the reads were mapped to the reference sequence of T-DNA (pDJW5) using CLC genomics workbench. T-DNA insertion sites were determined in all nine transformants with more than 10 forward and reverse reads containing *S. sclerotiorum*/T-DNA juncture sequences. The sequences of the inserted T-DNAs were often incomplete with boarder sequences missing. The sequences of *S. sclerotiorum* at the juncture were used as quarries to BLAST search the genome of *S. sclerotiorum*. The identified T-DNA insertion loci were further confirmed using PCR with appropriately designed primers. Together, our results show that the next-generation sequencing of pooled DNA samples is a highly cost-effective and fast method to determine the disrupted genes in T-DNA transformants of *S. sclerotiorum*..

429. Chartreusin inhibits appressorium formation of *Phytophthora infestans*. Shuji Tani[1], Masaya Kawamoto[1], Kenji Kai[1], Daisuke Hagiwara[2], Jun-ichi Sumitani[1], Takashi Kawaguchi[1]. 1) Life & Environmental Sci, Osaka Prefecture Univ, Osaka, Osaka, Japan; 2) MMRC, Chiba University, Chiba, Japan.

Infection of the oomycete *Phytophthora infestans* into plants is basically achieved through the differentiation steps of zoospore release from sporangia, cyst formation, and appressorium formation at cold temperatures. To help understanding of the relevant molecular mechanisms of the cold-induced development, we screened for the compounds from actinomycetes that exhibit stage specific inhibition of the sporangium development. Testing samples were prepared by mixing equal parts of acetone and actinomycetes culture. Sporangia with or without samples were kept at 10°C for 16 hours and observed morphological change under the microscopy. Among 800 samples tested, 2 samples inhibited specifically the appressorium formation, while these samples did not affect the vegetative growth on rye media at 18°C. The 16S rRNA gene sequences of both isolated strains showed significant similarities to that of *Streptomyces chartreusis*. By optimizing the compound producing condition, we selected the one strain, namely *Streptomyces* sp. no. 287, since it produced consistently more compounds than the other strain. *Streptomyces* sp. no. 287 was cultivated in liquid medium at 30°C with shaking to yield 8.7 liter culture broth. Equal volume of EtOAc was added to the culture broth to extract the active compounds, and which were applied for series of ODS column chromatography. We finally obtained 3 mg of the purified compound. The ESI-MS, ^{1}H-NMR, and ^{13}C-NMR spectra of the purified molecule proved that the purified molecule was identified as chartreusin. Biological activities of the purified molecule and chartreusin on the *P. infestans* sporangium development were also identical. When sporangia were treated with chartreusin, cyst germination was moderately inhibited. However, in case that cysts were treated with chartreusin, appressorium formation was inhibited. Furthermore, chartreusin significantly inhibited the infection of *P. infestans* 1306 to tomato leaves. Now, we are analyzing the effect of chartreusin on sporangium development at transcriptional level.

430. Conidia of the human pathogenic fungus *Aspergillus fumigatus* interfere with the maturation of macrophage phagolysosomes. Hella Schmidt, Andreas Thywißen, Axel Brakhage, Thorsten Heinekamp. Molecular and Applied Microbiology, Hans Knoell Institute, Jena, Germany.

Aspergillus fumigatus is a major cause for fungal infection in immunocompromised hosts. Conidia of this pathogen are ubiquitous in nature and enter the human host via the airway, where they infect the lung tissue and intrude to the lower respiratory system.

Invading conidia in the lung tissue are phagocytosed by alveolar macrophages and degraded in the phagolysosome. However, *A. fumigatus* prevents its killing by arresting the maturation of the phagolysosome. The pigment dihydroxynaphthalene (DHN)-melanin is crucial in this process.

Here, we aim to decipher in more detail the immune evasion strategy of *A. fumigatus* conidia from phagolysosomes. For that purpose, melanized wild-type conidia and non-melanized *pksP* mutant conidia were co-incubated with macrophages and the ratio of phagocytosis events and acidification of phagolysosomes was monitored. Furthermore, phagolysosomes containing conidia of both strains were purified from cell extracts. The proteome of the organelle was assessed by means of western blot analyses and liquid chromatograpy/mass spectrometry.

Conidia-containing phagolysosomes were purified by labeling the conidia with magnetic beads. Their identity was confirmed by the detection of specific phagolysosomal marker proteins. Proteome analysis revealed several candidates important in the maturation process.

We propose a mechanism by which conidial DHN-melanin interferes with the acquisition of a specific protein composition of phagolysosomes, hampering thereby the efficient intracellular clearance of invading *A. fumigatus* conidia.

431. Effector Biology in *Aspergillus fumigatus*. Allison Powell[1], Kelly Drews[1], Kelsey Simmons[1], Helen Clark[1], Hua Wise[1], Vincenzo Antignani[1], Heather Martinez[1], Brett Tyler[1], Shiv Kale[2]. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Oregon State University, Corvallis, OR.

Aspergillus fumigatus is a ubiquitous, saprophytic, filamentous fungus capable of causing a diverse array of disease manifestations in humans ranging from pronounced allergic responses to invasive infections. The role of effectors in facilitating infection is well established amongst several plant pathogenic fungi and oomycetes. Based on this understanding, we chose to examine the role of effectors during pulmonary invasive aspergillosis. Putative effector families were computationally identified through a clustering based algorithm. One such family conserved amongst several species of Aspergillus and clinically relevant fungi contained a N-terminal RxLR-like motif. The RxLR and RxLR-like signatures have been previously shown to facilitate effector delivery into plant and animal cells via binding cell surface PtdIns-3-P. Here we describe AF2, a candidate effector from *A. fumigatus* that contains a N-terminal RxLR-like motif. Using BEAS-2B cells we show recombinantly expressed and purified AF2 labeled with FITC is rapidly able to enter cells via PtdIns-3-P mediated endocytosis through binding by its RxLR-like motif. Recombinant AF2 was further characterized by isothermal titration calorimetry, and surface plasmon resonance to determine phospholipid binding, specifically to PtdIns-3-P. Knockout of AF2 in Af293 resulted in decreased fungal burden in murine models of leukopenia and neutropenia. The novel discovery of the role of effectors amongst clinically relevant fungi provides an opportunity to design therapeutics focused around blocking effector entry and targeting.

432. When Sex Meets Virulence - Scrutinizing the *Aspergillus fumigatus* Mating-Type Idiomorphs. Yidong Yu[1], Cornelia Will[1], Edyta Szewczyk[2], Jorge Amich[2], Sven Krappmann[1]. 1) Institute of Microbiology - Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nürnberg, Germany; 2) Research Center for Infectious Diseases, Julius-Maximilians University Würzburg, Germany.

Our studies aim at a comprehensive analysis of the extant sexuality that is displayed by the human-pathogenic mould *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) and its relevance for virulence. Sexual reproduction of *A. fumigatus* was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008. The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing the presence of mating-type idiomorphs (*MAT1-1* and *MAT1-2*) and of several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-2* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which reveals the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding factors presumably involved in this process. Comprehensive transcriptional profiling studies reveal the depth of the mating-type factor-directed transcriptomes. Functional analysis of a novel presumed mating-type gene associated with the *MAT1-2* idiomorph indicates its necessity for fruiting body formation, assigning the corresponding gene product a functional role in the mating process. Furthermore, analysis of a congenic pair of strains differing only in their mating-type identity is presented with the aim to systematically scrutinize any phenotypical differences, especially with respect to virulence, that may be based on the *MAT1* genotype.

433. Application of the high-throughput *Aspergillus fumigatus* cell wall-stress reporter system to identify synthetic peptides increasing the sensitivity for antifungal medicines. Cees van den Hondel[1,2], Ellen Lagendijk[1], Sophie Meier zu Ummeln[1], Christien Lokman[3], Arthur Ram[1]. 1) DLeiden University, Institute of Biology Leiden, Molecular Microbiology, Sylviusweg 72, 2333 BE Leiden, The Netherlandsept Mol Microbiol.,Inst Biol, Univ Leiden, Leiden, Netherlands; 2) HiTexacoat, Gouda, The Netherlands; 3) HAN

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Increased resistance to currently used antifungal compounds and the fact that these agents are often harmful to man and environment have resulted in a growing demand for new antifungals, which selectively act on cellular processes that are unique to fungi. To meet this demand, we have established in A. niger a luciferin/luciferase based reporter system for high-throughput screening of natural products for identification of potential new antifungal drugs). Our system allows us to identify compounds that specifically inhibited the fungal cell wall biosynthesis. Recently a similar system has been developed for A. fumigatus as well as for other non-Aspergillus species.

On our poster we will show the validation of our system by analysing its performance with several antifungal drugs which are commonly used in the clinic like Caspofungin, Amphotericin B, Voriconazol (V fend) as well as Nikkomycin. This analysis clearly shows not only a doses response behaviour of the compounds tested but also that in time different types of stress responses occur. Importantly, also the analysis shows a clear indication whether the compound tested is fungistatic or fungicidal.

Subsequent analysis of different synthetic antimicrobial peptides, HTX1-4, revealed also a moderate effect on cell wall stress induction indicated by an increase in Lux activity. Interestingly, incubation of these peptides at sublethal concentrations together with some of the antifungal medicines showed a considerable increase in lux activity and a significant increase in sensitivity of A.fumigatus for these medicines.

434. *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. Isabelle Benoit[1,2,3,4], Marielle van den Esker[5], Aleksandrina Patyshakuliyeva[1,2,3], Derek Mattern[6], Felix Blei[8], Miaomiao Zhou[1,3], Jan Dijksterhuis[1], Axel Brakhage[6,7], Oscar Kuipers[5], Ronald de Vries[1,2,3,4], Ákos Kovács[5,8]. 1) Fungal Physiology, CBS-KNAW, Utrecht, Utrecht, Netherlands; 2) Microbiology, Utrecht University, Utrecht, The Netherlands; 3) Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands; 4) Kluyver Centre for Genomics of Industrial Fermentations, Netherlands Genomics Initiative/Netherlands Organization for Scientific Research, Netherlands; 5) Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; 6) Leibniz Institute for Natural Product Research and Infection Biology, HKI, Jena, Germany; 7) Department of Microbiology and Molecular Biology, Institute of Microbiology, Friedrich Schiller University Jena, Jena, Germany; 8) Terrestrial Biofilms Group, Institute of Microbiology, Friedrich Schiller University Jena, Jena, Germany.

Interaction between microbes affects the growth, metabolism and differentiation of members of the microbial community. While direct and indirect competition, like antagonism and nutrient consumption have a negative effect on the interacting members of the population, microbes have also evolved in nature not only to fight, but in some cases to adapt to or support each other, while increasing the fitness of the community. *Bacillus subtilis*, when grown in the presence of *Aspergillus niger* interacts with the fungus by attaching and growing on the hyphae. Based on data obtained in a dual transcriptome experiment, we suggest that both fungi and bacteria alter their metabolism during this interaction. Interestingly, the transcription of genes related to the antifungal and putative antibacterial defense mechanism of *B. subtilis* and *A. niger*, respectively, are decreased upon attachment of bacteria to the mycelia. Analysis of the culture supernatantsuggests that surfactin production by *B. subtilis* was reduced when the bacterium was co- cultivated with the fungus. Our experiments provide new insights into the interaction between a bacterium and a fungus.

435. Dld1, a novel fungal histidine-rich effector protein, binds to metal ions to perturb plant immunity. R. Nostadt[1], M. Hilbert[1], J. Martin[2], U. Lahrmann[3], S. Nizam[1], A. Lupas[2], A. Zuccaro[1,4]. 1) Department of Organismic Interactions, MPI for Terrestrial Microbiology, Marburg, Germany; 2) Department of Protein evolution, MPI for Developmental Biology, Tübingen, Germany; 3) Fraunhofer Institute for Toxicology and Experimental Medicine, Research for human health, Regensburg, Germany; 4) Institute for Genetics, Biocenter Cologne, University of Cologne, Cologne Germany.

Our research is focused on understanding the basic mechanisms of establishment of mutualistic interactions between different plant hosts and their root endophytes. As model organism we use the sebacinoid fungus *Piriformospora indica*, a transformable, easily cultivable, growth promoting endophyte. It has the ability to colonize the root cortex of a wide range of plants inter- and intracellulary and increases tolerance against biotic and abiotic stresses. It is well known that microbial pathogens inhibit plant defense responses by producing small secreted proteins, so called effectors. Recently, it has been shown that such effector-like proteins play an important role in establishment of the mutualistic interactions. Genomic analysis of *P. indica* revealed the presence of a novel putative effector gene family encoding proteins with a regular distribution of histidine residues and a highly conserved C terminal motif ‘‘RSIDELD’’. We show here that one member of this family, Dld1, has the ability to bind metal ions at different pH values. Specifically Dld1 is able to bind iron(III) with high affinity. Upon *P. indica* colonization of barley roots the host reacts with targeted redistribution of iron(III) to the site of attempted penetration where production of cell wall appositions and redox activity is observed. The bulk secretion of iron(III) and its accumulation at penetration sites has been shown to mediate the oxidative burst and regulate defense responses in cereals. By a cytological and biochemical approach we demonstrate that Dld1 distribution coincides with that of iron(III) at the host cell wall appositions in barley. Additionally this effector is able to directly suppress the iron(III) dependent generation of ROS in a concentration-dependent manner, suggesting a role in perturbation of cereal host immunity.

436. Identifying interactors of RNase like effector BEC1054. Helen G Pennington[1], Rhian Jones[1], Dana M Gheorghe[1], Ernesto Cota Segura[1], Rainer Cramer[2], Laurence V Bindschedler[2,3], Pietro D Spanu[1]. 1) Life Sciences, Imperial College London, London, United Kingdom; 2) Chemistry, University of Reading, Reading, United Kingdom; 3) School of Biological Sciences, Royal Holloway University of London (RHUL), Egham, United Kingdom.

The fungus *Blumeria graminis* f. sp. *hordei*, is a biotrophic, economically important fungal pathogen which infects barley, causing powdery mildew disease. The fungus utilises ‘secretory warfare’ to compromise host immunity, with effectors being delivered at the

haustorial complex. One such effector, *Blumeria* Effector Candidate 1054 (BEC1054), is predicted to be a ribonuclease like protein. Effector function has been verified for this BEC (Pliego et al., 2013). A recombinant BEC1054 protein with an N-terminal His tag was expressed in *E.* coli, purified, and used as the bait to identify putative interacting proteins from barley leaf epidermal material, and from barley leaf material through *in vitro* liquid chromatography mass spectrometry. A total of 247 proteins were identified interacting solely with BEC1054, when compared with an unrelated BEC pull-downs or negative controls. Within these 247 proteins, small 40S ribosomal subunit proteins and eukaryotic elongation factors (eEF)s were significantly overrepresented; with eEFs being overrepresented in nearly all experimental conditions used. The interaction of a number of these with BEC1054 was investigated through yeast-two-hybrid. Two, a glutathione-S-transferase (GST) and a PR5 protein were found to interact weakly in two bait-prey orientations. Furthermore, elongation factor 1 gamma, a malate dehydrogenase (MDH), and a ribosomal subunit 40S 16 protein interacted in one bait-prey orientation. Three interactions, a PR5, a GST and an eEF1a have further been validated in the host plant, barley via Bimolecular Fluorescence Complementation (BiFC). These results, and literature searches, have allowed us to create a model for BEC1054's interaction with the ribosome. We hypothesise that BEC1054 outcompetes Ribosome Inactivating Proteins (RIP)s, helping to prevent the hypersensitive response, and thus allowing the biotrophic interaction to continue.

437. Plant defence hormone sensing in the biotrophic fungus *Ustilago maydis*. F. Rabe[1], D. Seitner[1], A. Czedik-Eysenberg[1], R. Kahmann[2], A. Djamei[1]. 1) Gregor Mendel Institute of Molecular Plant Biology, Dr. Bohr-Gasse 3, 1030 Vienna, Austria; 2) Max Planck Institute of Molecular Microbiology, Karl-von-Frisch-Strabe 10, 35043 Marburg, Germany.

The plant pathogenic fungus *Ustilago maydis* causes smut disease on its host plant *Zea mays*. Upon recognition of Pathogen associated molecular pattern plants induce local and systemic defence responses leading to systemic acquired resistance. In order to establish the biotrophic interaction the pathogen secretes hundreds of small proteins, so called effectors to suppress plant defence responses and to re-channel the host metabolism. Salicylic acid (SA) is a key plant defence hormone in response to biotrophic pathogens. Due to its importance for host defence it could be directly targeted for degradation by biotrophic pathogens but it could also be a measure for the defence status of its host. Recently we reported the identification of a functional Salicylate hydroxylase that is transcriptionally induced during the biotrophic phase of the fungus and is essential for degradation of the plant defence hormone Salicylic acid by *Ustilago maydis* (Rabe et al 2013). Here we report the identification of a key component of the SA sensing mechanism in *Ustilago maydis*. A genetic screen led to the identification of the SA-Response Factor SARF1. SARF1 is a nuclear localized protein and is constitutively expressed. Our results indicate that SARF1 could be the first reported fungal Salicylic acid receptor.

438. Specific expression of candidate effectors of the rust fungus *Melampsora larici-populina* during infection of its two host plants, larch and poplar. Sebastien Duplessis[1,2], Stephane Hacquard[1,2,3], Antoine Persoons[1,2], Christine Delaruelle[1,2], Jeremy Petrowski[1,2], Pascal Frey[1,2], Benjamin Petre[1,2,4]. 1) INRA, UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, 54280 Champenoux, France; 2) Université de Lorraine, UMR 1136 INRA/Université de Lorraine Interactions Arbres/Microorganismes, 54280 Champenoux, France; 3) Present address, Max Planck Institute for plant breeding research, Köln, Germany; 4) Present address, The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.

Melampsora larici-populina (Basidiomycete, Pucciniales) is one of the rust fungi responsible for the poplar leaf rust disease. It has a complex macrocyclic and heteroecious life cycle, marked by the production of five different spore forms in two different host plants: larch (sexual reproduction) and poplar (asexual clonal reproduction). The asexual stage leads to severe rust epidemics recorded in poplar plantations. As for other rust fungi, the asexual stage has been well covered and characterized, whereas we have almost no knowledge of other stages of the life cycle. Particularly, the capacity for the fungus to infect hosts with distinct taxonomical positions raises questions about the molecular bases underlying the specificity of host-rust interactions. Following the genome sequencing of *M. larici-populina* isolate 98AG31, the secretome annotation has revealed a large repertoire of nearly 1200 genes encoding small secreted proteins and expression profiles of these candidate rust effectors have been defined by oligoarray-based transcriptomics during poplar infection. We present here the transcriptome analysis by RNA-Seq (Illumina) of three fungal structures associated to the infection and reproduction stage on larch: basidia, spermogonia and aecia. These were obtained following an original system for controlled infection in laboratory conditions using the reference isolate 98AG31. Almost 300 millions reads were generated for each condition (three biological replicates) and were used to show specific expression profiles in the larch host. Comparison with the profiles previously obtained in poplar reveals the presence of specific sets of candidate effectors expressed either in each or in both host plants.

439. *Thecaphora thlaspeos,* a smut pathogen that colonizes the model plant *Arabidopsis thaliana*. Lamprinos Frantzeskakis[1], Kaitlyn Courville[1], Sabrina Baltes[1], Ronny Kellner[2], Andreas Brachmann[3], Michael Feldbrügge[1,4], Vera Göhre[1,4]. 1) Institute for Microbiology, Heinrich-Heine University, Düsseldorf, Germany; 2) The Sainsbury Laboratory, Norwich, UK; 3) Faculty of Biology, Genetics, University of Munich (LMU), Martinsried, Germany; 4) Cluster of Excellence on Plant Sciences (CEPLAS).

Smut fungi are biotrophic plant pathogens that infect economically important crops. Well-characterized species of this group, such as *Ustilago maydis*, *U. hordei* and *Sporisorium reilianum* are host-specific and only able to infect grasses. A limitation of these established pathosystems is that the hosts are not easily genetically modified and their responses cannot be readily studied.

To overcome this limitation and investigate the behavior of biotrophic fungal pathogens in dicot plants, we are following an alternative approach. We have collected isolates of the smut fungus *Thecaphora thlaspeos*, which infects perennial Brassicaceae species, such as *Arabis hirsuta.* During our collections, for four subsequent years in several locations in Europe, we have identified a novel host, *A. ciliata*. *T. thlaspeos* grows endophytically and presents symptoms only in the final stages of the plant development, by producing spores in the growing siliques.

Here we report the draft genome of *T. thlaspeos* and further experimental work, which shows that although a tetrapolar mating system exists, mating is not necessary for filamentation as in other smut fungi. Currently, we are continuing the analysis and manual annotation of the *T. thlaspeos* genome in order to describe the metabolic capacity and infection toolbox of this pathogen.

Interestingly, initial experiments show that *T. thlaspeos* is able to colonize *Arabidopsis thaliana*. This opens up the possibility of using *T. thlaspeos* and *A. thaliana*, as two genetically tractable partners in a new pathosystem, to study fungal effectors and host responses.

440. Identification of seedling-specific effectors:From organ to cell-type specificity. Alexandra Matei[1,2], Amey Redkar[2], Lena Schilling[2], Virginia Walbot[3], Gunther Doehlemann[1,2]. 1) Botanical Institute and Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, BioCenter, Zuelpicher Str. 47a, 50674 Cologne, Germany; 2) Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strabe 10, 35043 Marburg; 3) Department of Biology, Stanford University, MC5020 385 Serra Mall, Stanford, CA 94305-5020, USA.

The basidiomycete fungus *Ustilago maydis* is a soil-borne fungus that causes smut disease on maize. It is the only smut that can infect all aerial organs of the maize plant. The fungus therefore deploys a highly adaptable set of effector proteins to circumvent host immunity and induce tumor-formation in different types of host tissue. In order to understand the organ-specificity of *U. maydis*, a set of effector proteins with organ-specific virulence functions was identified on the basis of the preliminary transcriptome analysis. The functional analysis of these *U. maydis* effector genes demonstrated that fungal effectors function in an organ-specific manner.

To understand how tissue-specific effectors contribute to fungal virulence, the seedling-specific effector STS1 was analyzed for its interaction partner by yeast-II-hybrid analysis. This identified a maize carboxypeptidase which is described to be involved in growth control and development as well as carbon and amino acid metabolism in plants as a putative interactor of STS1. Currently, interaction of STS1 with its host target is tested via Co-immunoprecipitation and fluorescence complementation *in-vivo*. In a next step, we will investigate the biological relevance of the observed interaction for leaf-specific tumor progression.

In a second approach, we aim to elaborate the tissue- and cell-type specific activity of *U. maydis* effector proteins. To this end, a cell-type specific gene expression analysis together with *in situ* hybridization experiments is conducted. This combined approach aims to visualize interactions of fungal and host factors in a cell-type specific and temporal context.

441. The *flp1* and *flp2* genes encode fasciclin I-like proteins that contribute respectively to early or late infection of plant by *Botrytis cinerea*. Jordan Ferria, Christine Rascle, Pavle Smilevski, Gwenlyn Fleury, Christophe Bruel, Nathalie Poussereau, Mathias Choquer. Université Lyon 1, CNRS, BAYER SAS: 14 impasse Pierre Baizet, BP 99163, 69263 Lyon Cedex 09, France.

Type I Fasciclins are secreted or membrane-anchored proteins that display cell adhesion properties by homophilic interactions in a wide-range of eukaryotic organisms. In fungi, two fasciclin I-like proteins (Flp) are usually found but their biological role is poorly understood. Inactivation of the *flp1* gene in *Magnaporthe oryzae* led to significant decrease in conidiation, conidial adhesion, appressorium turgor and pathogenicity (Liu *et al*., 2009). In *Fusarium graminearum*, *AurS* gene (*flp2*) was proposed as a member of an extracellular enzyme complex responsible for the biosynthesis of Aurofusarin polyketide (Frandsen *et al*., 2011). In order to determine whether fasciclin I-like proteins contribute to virulence in the plant-pathogen fungus *Botrytis cinerea*, Δ*flp1* and Δ*flp2* simple mutants were constructed and analyzed. Mutants seem unaffected in their radial growth on poor or rich media but are altered in their sporulation. Inoculation of mycelial plugs on gherkin cotyledons showed that Δ*flp1* mutant is still pathogenic but display a significant delay in the appearance of primary lesion. Flp1 protein could be implicated in fungal penetration as this defect can be overcome by wounding the plant. The most dramatic phenotype was observed for the Δ*flp2* mutant that is completely stopped during the plant colonization process. *In vitro* qRT-PCR expression profiles were in agreement with these observations as *flp1* gene is overexpressed immediately after conidial germination and *flp2* gene is overexpressed in aged mycelium. As fungal growth was mainly affected *in planta*, we propose that Flp1 and Flp2 proteins could be early and late virulence factors in *B. cinerea*, respectively. The double mutant Δ*flp1*/Δ*flp2* has been constructed in order to study putative interactions between genes.

Liu *et al.* (2009) Journal of Zhejiang University Science B. 10: 434-44.

Frandsen *et al.* (2011) Journal of Biological Chemistry. 286: 10419-28.

442. Pre-adaptation to host microenvironments impacts *Candida albicans*-host interactions during infection. Elizabeth Ballou[1], Joanna Potrykus[1], Yohann Coute[2], David Stead[1], Donna MacCallum[1], Myriam Ferro[2], Alistair Brown[1]. 1) College of Life and Medical Sciences, Institute of Medical Sciences, Aberdeen, United Kingdom; 2) CEA, DSV, Université Grenoble Alpes, FR.

Here we investigate host-fungal interactions in the context of host nutrient availability. The human fungal pathogen *Candida albicans* resides in glucose-poor microenvironments both as a gut commensal and as a systemic pathogen. Previously, we showed that glucose limitation and growth on the physiologically relevant carbon source lactate alters the cell wall and secretome, shifts cytokines from protective Th17 to anti-inflammatory Th2 profiles and increases stress resistance. Lactate-grown *C. albicans* are also more resistant to phagocyte killing and cause more severe systemic disease. Previous studies of *C. albicans*-phagocyte interactions revealed a metabolic shift upon engulfment, consistent with nutrient limitation within phagocytes. However, our data suggest that, more than simply altering metabolic profiles, carbon source also impacts signal transduction and stress response, effectively priming *C. albicans* to interact with the immune system. To investigate the role of carbon source in host-pathogen interaction, we performed label free quantitative mass

spectrometry of the whole proteome of *C. albicans* grown in either glucose or lactate and then co-cultured for 2 hours with or without human neutrophils. The resulting 4-way comparison differentiated proteins involved in carbon source adaptation from those specifically involved in neutrophil response. The former is typified by the oxidative stress resistance catalase Cat1, which was more abundant in lactate- than glucose-grown cells. Following co-incubation with neutrophils, Cat1 abundance moderately increased in glucose but not lactate cells, consistent with pre-adaptation of lactate-grown cells to oxidative stress. In contrast, proteins such as the adhesin Als3 and the mannosyl-transferase Mnt1 were not impacted by carbon source alone but appeared to be differentially repressed by incubation with neutrophils in a carbon source-dependent manner. We have investigated these trends using transcriptional and mutant analysis to reveal networks underlying the impact of host microenvironment on *C. albicans* pathogenesis.

443. The exportin Nmd5 modulates intracellular level of the iron-responsive regulator Sef1 and prevents inappropriate gene activation in the human fungal pathogen *Candida albicans*. Changbin Chen[1], Xinhua Huang[1], Suzanne Noble[2]. 1) Unit of Pathogenic Fungal Infection & Host Immunity, Institut Pasteur of Shanghai, Shanghai, China; 2) Dept of Microbiology & Immunology, UCSF, San Francisco, CA, USA.

Pathogenic fungi, like bacterial pathogens, must capture iron from host tissues and regulate iron homeostasis to avoid toxicity. In the opportunistic human fungal pathogen *Candida albicans*, we previously discovered that Sef1, a Zn_2Cys_6 zinc knuckle transcriptional factor, regulates iron homeostasis by integrating into a highly conserved regulatory circuit with reciprocal roles in *C. albicans* commensalism and virulence[1]. Post-transcriptional regulation of Sef1, including phosphorylation and nuclear localization, determines its role in pathogenesis[2]. Here we present evidence that Nmd5, encoding a putative exportin, acts to balance intracellular level of Sef1 by regulating its cytoplasm-nuclear localization. *NMD5* overexpression mislocalizes Sef1 to cytoplasm whereas its deletion does not interfere with Sef1 nuclear localization, indicating that Nmd5 exports Sef1 to cytoplasm. Compared to the wild type, *NMD5* deletion causes an even higher level of Sef1 protein but not RNA, and the relevant strain shows resistance to iron depletion. However, the excessive Sef1 in the *nmd5*Δ/Δ mutant did not hyper-activate expression of iron uptake genes and our RNA-Seq results suggest that in low iron condition, Nmd5 exports excessive Sef1 to cytoplasm and thereby prevents any inappropriate gene induction. Together, we identified a novel role of Nmd5 in transporting Sef1 protein: an exportin to export Sef1 to cytoplasm under low iron condition, and this prevents cryptic induction of gene transcription by Sef1 and specifies its role in promoting virulence.

1. ***Chen, C*.***, Pande, K*., French, S. D., Tuch, B. B. and Noble, S. M. (2011) ***Cell Host & Microbe***. 10(2): 118-35. (*equal contribution)

2. ***Chen, C.*** and Noble, S. M. (2012). ***PLOS Pathogens***, 8(11): e1002956.

444. *Candida albicans OPI1* regulates filamentous growth and virulence in vaginal infections, but not inositol biosynthesis. Y. Chen[1], F. de Bernardis[2], S. Yu[1], S. Sandini[2], S. Kauffman[3], R. Tams[3], E. Bethea[3], T. Reynolds[3]. 1) Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan; 2) Department of Infectious, Parasitic and Immunomediated Diseases, Rome, Italy; 3) Department of Microbiology, University of Tennessee, TN, USA.

ScOpi1p is a well-characterized transcriptional repressor and master regulator of inositol and phospholipid biosynthetic genes in the baker's yeast *Saccharomyces cerevisiae*. An ortholog has been shown to perform a similar function in the pathogenic fungus *Candida glabrata*, but with the distinction that CgOpi1p is essential for growth in this organism. However, in the more distantly related yeast *Yarrowia lipolytica*, the *OPI1* homolog was not found to regulate inositol biosynthesis, but alkane oxidation. In *Candida albicans*, the most common cause of human candidiasis, its Opi1p homolog, CaOpi1p, has been shown to complement a *S. cerevisiae opi1Δ* mutant for inositol biosynthesis regulation when heterologously expressed, suggesting it might serve a similar role in this pathogen. This was tested in the pathogen directly in this report by disrupting the *OPI1* homolog and examining its phenotypes. It was discovered that the *OPI1* homolog does not regulate *INO1* expression in *C. albicans*, but it does control *SAP2* expression in response to bovine serum albumin containing media. Meanwhile, we found that CaOpi1 represses filamentous growth at lower temperatures (30°C) on agar, but not in liquid media. Although, the mutant does not affect virulence in a mouse model of systemic infection, it does affect virulence in a rat model of vaginitis. This may be because Opi1p regulates expression of the *SAP2* protease, which is required for rat vaginal infections.

445. *Candida albicans* quorum sensing molecule stimulates the migration of innate immune cells in vivo. Jessica C. Hargarten[1], Tyler C. Moore[1,2], Deborah M. Brown[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.

The polymorphic commensal fungus *Candida albicans* causes life-threatening disease during bloodstream infections. Although host immune evasion is a common strategy employed by successful human pathogens, including *C. albicans*, we hypothesize that particular morphologies of *C. albicans* may stimulate immune recognition by host innate immune cells whose defenses are incapable of controlling fungal virulence on their own. *C. albicans* white cells secrete a small molecular weight macrophage chemo-stimulant and are able to survive within and escape from macrophages, while opaque cells do not. One likely candidate is farnesol because opaque cells, unlike white cells, do not produce 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 in mouse macrophages. The production of these cytokines by macrophages is an important indicator of whether the immune response can clear 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 that *E,E*-farnesol is a potent stimulator of macrophage movement both in vitro and in vivo, an activity enhanced by yeast cell wall components and aromatic alcohols. Intraperitoneal injection of farnesol recruits and activates a temporally distinct pattern of innate immune cells to the peritoneal cavity of mice. Modulation of farnesol secretion to stimulate host immune recognition by ineffective phagocytes may help explain why this commensal is such a successful

pathogen in immunocompromised individuals.

446. Iron adaptation mechanisms by the pathogenic yeast *Candida albicans* are responsive to a bacterial quorum sensing molecule. F. Hennicke[1,2,6], S. Brunke[3], B. Hube[3], C. Staib[4], P. Staib[5,6]. 1) Department of Mycology, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany; 2) LOEWE Excellence Cluster for Integrative Fungal Research (IPF), Frankfurt am Main, Germany; 3) Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Jena, Germany; 4) Department of Obstetrics and Gynecology, University of Würzburg, Würzburg, Germany; 5) Research and Development, Kneipp GmbH, Würzburg, Germany; 6) Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Jena, Germany.

Little is known on interkingdom signalling between pathogenic fungi and co-infecting bacteria. Here, we examined whether the human pathogenic yeast *Candida albicans* can specifically respond to a low physiological concentration of 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), a quorum sensing molecule from the Gram-negative bacterium *Pseudomonas aeruginosa*. Focussing on selected *C. albicans* genes involved in iron adaptation mechanisms, we measured their transcriptional response to 3-oxo-C12-HSL. Subsequently, *C. albicans* yeast growth was examined under iron-limited conditions after pre-exposure to 3-oxo-C12-HSL. Monitoring the transcriptional response of *C. albicans* genes involved in iron adaptation, a specific activation of key factors from high-affinity iron acquisition systems was detected. At the same time, factors involved in iron consuming processes were found to be repressed. In accordance to these observations, growth experiments evidenced that *C. albicans* cells, which were pre-exposed to 3-oxo-C12-HSL, showed an increased potential to adapt to iron starvation conditions. These findings provide novel insights into the molecular basis of host adaptation mechanisms in *C. albicans*, and moreover exemplify the interspecies crosstalk between fungi and bacteria.

447. Global Analysis of Fungal Morphology Exposes Mechanisms of Host Cell Escape. Teresa O'Meara[1], Amanda Veri[1], Troy Ketela[1], Bo Jiang[2], Terry Roemer[2], Leah Cowen[1]. 1) Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Merck Research Laboratories, New Jersey, United States.

Developmental transitions between single-cell yeast and multicellular filaments underpin virulence of diverse fungal pathogens. For the leading human fungal pathogen *Candida albicans*, filamentation is thought to be required for immune cell escape via induction of an inflammatory programmed cell death. We performed genome-scale analysis of *C. albicans* morphogenesis in response to host-relevant cues and identified 879 morphogenetic regulators, with key roles for ergosterol biosynthesis and N-linked glycosylation. We discovered that *C. albicans* filamentation is not required for escape from host immune cells, and that macrophage pyroptosis is driven by cell wall remodeling and exposure of glycosylated proteins in response to the macrophage phagosome. The capacity of killed, previously phagocytized cells to drive macrophage lysis was observed with the distantly related fungal pathogen *Cryptococcus neoformans*. This study provides a global view of morphogenetic circuitry governing a key virulence trait, and illuminates a new paradigm by which fungi trigger host cell death.

448. Dissecting the role of metal depletion in *C. albicans* echinocandin resistance and morphogenesis. Elizabeth Polvi, Leah Cowen. Dept Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Candida albicans prevails as one of the leading causes of invasive fungal infections worldwide, causing severe systemic infections in immunocompromised individuals. Echinocandins inhibit synthesis of a structural polysaccharide in the fungal cell wall and resistance to this newest class of antifungal has already emerged. Resistance to echinocandins is mostly caused by mutations in the drug target gene, *FKS1*. Additionally, cellular stress responses are required to mediate resistance acquired by drug target mutations but additional mediators of echinocandin resistance remain to be discovered. Here, we utilize a pharmacological screen to identify novel circuitry that regulates resistance to echinocandins and to find compounds that can be used in combination with echinocandins to combat resistance. We screened a library of 1280 pharmacologically active compounds, and identified four that reduce the echinocandin resistance of a clinical isolate. To further characterize these compounds, we tested their effects on *C. albicans* morphogenesis, as the ability to transition between yeast and filamentous states is a key virulence trait. We focused on a chelator, DTPA, which we found to both abrogate echinocandin resistance and induce filamentation. Depletion of magnesium abrogates echinocandin resistance suggesting that DTPA chelates magnesium to confer sensitivity to the echinocandin caspofungin. To further investigate the mechanism by which DTPA acts in combination with caspofungin, we selected for resistant mutants and performed whole genome sequencing. We identified mutations in a histidine kinase, *NIK1*, and are confirming that these mutations in *NIK1* are sufficient to confer resistance to both caspofungin and DTPA. We identified zinc depletion to be the relevant filament-inducing cue. Additionally, screening of a *C. albicans* library of transcription factor deletion mutants revealed that mutants lacking either Brg1 or Rob1 are unable to filament in response to DTPA. Testing additional mutants revealed that DTPA might influence the Nrg1-degradation pathway, and we are currently exploring the circuitry through which Brg1 and Rob1 influence responses to DTPA and zinc depletion.

449. Ecology of candidemia in pediatric small bowel transplant recipients. Mallory Suhr[1], Diana Florescu[2], David Mercer[3], Heather Hallen-Adams[1]. 1) Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE; 2) Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE; 3) Department of Surgery, University of Nebraska Medical Center, Omaha, NE.

Small bowel transplantation (SBT) is a life-saving medical procedure for patients with short bowel syndrome. However, these patients remain at high risk for bloodstream infections, with up to 59% of recipients developing one or more infections following transplant. In addition to numerous risk factors, these patients are unique by having two distinct microbial communities - those of the host and of the donor bowel. This research aims to characterize the SBT recipient gut microbiota over time following transplant and investigate the epidemiology of candidemia in pediatric SBT recipients. DNA was extracted from 28 ileostomy samples from 7 recipients (1-9 samples per patient) prior to, concurrent with and following candidemia. *Candida* species from the recipient gut and bloodstream were identified by ITS sequence and identified to strain by RAPD and MLST. Twenty-one ileostomy samples harbored at least 1 *Candida* sp. (*C. albicans, C.*

glabrata, C. parapsilosis, C. tropicalis). Bloodstream infections (9 isolates; 1 or 2 per patient) were caused by *C. parapsilosis* (4), *C. albicans* (2), *C. glabrata* (1), *C. orthopsilosis* (1), and *Wickerhamomyces anomalus* (1). Preliminary results show the same species present in ileostomy samples preceding infection and in bloodstream in at least three patients. Two patients had distinguishable ileostomy isolates from corresponding blood isolates at the strain level and one patient had indistinguishable ileostomy and blood isolates. Thus far, results are congruent with at least two infections arising from *Candida* other than that colonizing the recipient GI tract (*C. albicans, C. parapsilosis*) and one infection arising from the recipient GI tract (*C. glabrata*). Knowing the source of *Candida* implicated in infection (recipient, donor, or hospital acquired) will inform pre-transplant interventions to improve outcomes for SBT patients.

450. *Colletotrichum orbiculare* regulates cell cycle G1/S progression *via* two component GAP and GTPase to establish plant infection. Fumi Fukada, Ayumu Sakaguchi, Yasuyuki Kubo. Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto Prefectural University, Kyoto, Japan.

Morphogenesis in filamentous fungi depends on accurate cell cycle progression. Here, we report that cells of the cucumber anthracnose fungus *Colletotrichum orbiculare* undergo G1/S progression *via* two-component GAP CoBub2/CoBfa1 and its GTPase CoTem1 as they infect host plants. We performed a random insertional mutagenesis screen to identify genes involved in infection-related morphogenesis in *C. orbiculare*. In this screen, we isolated a homolog of *Saccharomyces cerevisiae BUB2*, which encodes a Rab GAP superfamily that negatively regulates the exit from mitosis making complex with Bfa1 when there is DNA damage, spindle damage or spindle misorientation at G2/M phase. Interestingly, mutations in the *C. orbiculare bub2* and *bfa1* accelerated the timing of nuclear division during appressorium development. Arresting the cell cycle from the onset of conidial incubation, the transition period from G1 phase to S phase was significantly accelerated in *Δcobub2* and *Δcobfa1*, suggesting these genes are involved in G1/S phase progression in *C. orbiculare*. The small GTPase *CoTEM1* is the homolog of *S. cerevisiae TEM1* which is controlled by Bub2/Bfa1 GAP complex and initiate the mitotic exit in *S. cerevisiae*. The phenotype of transformants that express dominant negative form *CoTEM1* in *Δcobub2* and *Δcobfa1* complemented the defect in G1/S progression, indicating that CoBub2/CoBfa1 regulates G1/S progression *via* CoTem1. Furthermore, pathogenicity assay revealed the *Δcobub2* and *Δcobfa1* developed appressoria but exhibited a specific defect in the subsequent host penetration step. The septin- and actin-assembly at the appressorium pore were attenuated, and more intense plant defense response was induced by *Δcobub2* and *Δcobfa1*. From these results we conclude that CoBub2/CoBfa1 coordinates G1/S progression *via* CoTem1 during appressorium development that are essential for establishment of plant infection.

451. Variability of *Colletotrichum* spp. in common bean. S. F. Mota, M. A. Dias, Q. L. Barcelos, E. A. Souza. Universidade Federal de Lavras, Brazil.

The *Colletotrichum* genus presents a large genetic variability among and within species. Anthracnose is important disease in worldwide and scab is emergent disease in common bean and both diseases are caused by species of the genus *Colletotrichum*, asexual form, and *Glomerella*, sexual form. *C. lindemuthianum* and *Colletotrichum* spp. isolates recovered from anthracnose and scab lesions in common bean have been characterized by pathogenic, morfological, cytological and DNA markers analysis. The relation between these species of the *Colletotrichum* genus, which inhabit anthracnose and scab lesions, have been discussed. The *Colletotrichum* spp. strains presented wide variability to all the evaluated traits, showing the occurrence of multiple species. Pathogenicity tests have showed that severity of anthracnose and scab should be evaluated in different time in common bean plant after inoculation. Only the *C. lindemuthianum* strains were grouped by the IRAP markers. Relevant informations were generated in this study that can contribute to basic and applied studies in the future to control of these diseases in common bean.

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452. A role of AREB in the Regulation of PACC-Dependent Acid-Expressed-Genes and Pathogenicity of *Colletotrichum gloeosporioides* . Dana Ment[1], Noam Alkan[1], Luria Neta[1], Fang-Cheng Bi[1], Robert Fluhr[2], Dov Prusky[1]. 1) Department of Postharvest Science, Agricultural Research Organization, Bet Dagan, Israel; 2) Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel.

Gene expression regulation by pH in filamentous fungi and yeasts is controlled by the PACC/RIM101 transcription factor. In *Colletotrichum gloeosporioides* PACC is known to act as positive regulator of alkaline-expressed genes, and this regulation was shown to contribute to fungal pathogenicity. However, PACC is also a negative regulator of acid expressed genes, but the mechanism of down-regulation of acid-expressed genes by PACC and their contribution to *C. gloeosporioides* pathogenicity is not well understood. RNA sequencing data analysis generated in previous publications, was employed to demonstrate that PACC transcription factor binding sites (TFBSs) are significantly over-represented in the promoter of PACC-up-regulated, alkaline-expressed genes. In contrast, they are not over-represented in the PACC-down-regulated, acid-expressed genes. Instead, acid-expressed genes showed over-representation of AREB GATA TFBS in *C. gloeosporioides* and in homologs of five other ascomycetes genomes. The *areB* promoter contains PACC TFBS, its transcript was up-regulated at pH 7 and repressed in *ΔpacC*. Furthermore, acid-expressed genes were found to be constitutively up-regulated in *ΔareB* during alkalizing conditions. The *areB* mutants showed significantly reduced ammonia secretion and pathogenicity on tomato fruits. Present results indicate that PACC activates *areB* expression, thereby conditionally repressing acid-expressed genes, and contributing critically to *C. gloeosporioides* pathogenicity.

453. Localization of *Colletotrichum higginsianum* putative effector proteins *in planta*.. Guillaume Robin, Richard O'Connell. BIOGER, INRA/AgroParisTech, THIVERVAL GRIGNON, France.

Colletotrichum higginsianum causes anthracnose disease on cruciferous plants, including *Arabidopsis*. The fungus uses a hemibiotrophic infection strategy, involving the formation of several specialized cell types. After melanized appressoria puncture host surfaces, bulbous biotrophic hyphae develop inside living host cells, surrounded by a modified host plasma membrane; finally the fungus switches to

destructive necrotrophy, associated with thin filamentous hyphae. The *C. higginsianum* genome encodes 365 putative effectors (ChECs), defined as predicted secreted proteins either with no homology to proteins outside the genus or resembling known effectors from other pathogens. Deep-sequencing of the *C. higginsianum* transcriptome during infection (Kleemann *et al*. 2012) and genome-wide expression analysis (O'Connell *et al.* 2012) revealed a set of 97 plant-induced effectors that are expressed in appressoria before host penetration and/or in biotrophic hyphae after penetration. This project aims to understand the role of these effectors in the early establishment of infection. Clues to effector function can come from knowing their destination inside host cells. We used *Agrobacterium tumefaciens* to transiently express 65 ChECs as N-terminal fusions with GFP in *Nicotiana benthamiana* leaf cells, revealing different patterns of subcellular localization. Nine ChECs are targeted to the plant nucleus, among which 3 specifically accumulate in the nucleolus, while 7 other ChECs localize to plant microtubules or organelles. To characterize effector functions, selected candidates are being expressed in transgenic *Arabidopsis* plants under 35S or dexamethasone-inducible promoters to investigate effects on plant immune responses, and their contribution to fungal virulence will be tested by targeted gene disruption in *C. higginsianum*.

Kleemann et al. 2012 PLoS Pathogens 8: e1002643

O'Connell et al. 2012 Nature Genetics 44: 1060-1067.

454. Functional characterization of CgEP2, a broadly conserved fungal effector with a role in virulence in *Colletotrichum graminicola*. José M. Sanz-Martín, Walter Vargas, Vinicio Armijos-Jaramillo, Michael R. Thon, Serenella A. Sukno. Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Department of Microbiology and Genetics, University of Salamanca, 37185 Villamayor, Spain.

During infection process, fungal pathogens secrete a wide range of enzymes and effector proteins to interact with their hosts and manipulate the plant immune system. To resist pathogen invasion, plants induce a large battery of defenses including PR proteins production such as chitinases. Recently, several fungal effectors have been identified that interfere with chitin-triggered immunity, protecting the fungal hyphae against hydrolysis by chitinases or sequestering the chitin oligosaccharides and preventing chitin from binding to the receptor. In this study, we describe CgEP2 (*Colletotrichum graminicola* Effector Protein 2), a 640 aa secreted protein in the maize pathogen *C. graminicola*, with a role in virulence. CgEP2 is a Zn dependent metalloprotease of the fungalysin family. Members of this family have been shown to bind to plant produces class IV chitinases (PR-4) and induce post-translational modifications. Phylogenetic analysis shows that CgEP2 is, highly conserved in diverse pathogenic fungi. Quantitative PCR (qPCR) assays using different time points during leaf anthracnose as well as transcriptional fusions of the gene promoter with a GFP cassette show that gene expression is activated at the late biotrophic stage, specifically when the fungus switches to necrotrophic growth. To confirm it's role in pathogenesis, we constructed null mutants tagged with GFP by gene replacement using Delsgate methodology and performed pathogenicity assays in maize. The null mutant develops reduced lesion sizes on leaves and reduced colonization of roots, showing that CgEP2 has a role in *C. graminicola* virulence. Also, biochemical analysis of chitinase activity in leaves infected with null mutant confirms the lack of ability to degrade this substrate. Our results show that CgEP2, which is a broadly conserved fungal effector, plays a role in plant infection and host colonization and could be important in the lifestyle change of *C. graminicola*.

455. Molecular and cellular analysis of the pH response transcription factor PacC in the fungal symbiont *Epichloe festucae*. Y. Lukito[1], T. Chujo[1,2], B. Scott[1]. 1) Institute of Fundamental Science, Massey University, Palmerston North, New Zealand; 2) Divison of Plant Sciences, National Institute of Agrobiological Sciences, Japan.

The role of the PacC transcription factor has been studied in a wide range of host-pathogen systems, and is known to regulate fungal pathogenicity and secondary metabolism. However, there are no reports to date of its role in a fungal-plant symbiotic interaction. We have examined the culture and plant interaction phenotypes of null and constitutively active mutations of *pacC* in *Epichloë festucae*, a mutualistic fungal symbiont of *Lolium perenne*. Colonies of the *pacC* null mutant had a premature senescence phenotype and were more sensitive to salt stress than wild-type. Relative expression levels of *enaA*, which encodes a putative Na^+-ATPase pump, were reduced in Δ*pacC* compared to wild-type. In contrast, expression of *enaB*, encoding a second isoform of the Na^+-ATPase pump, and *nhaA*, encoding a putative acid dependent H^+/Na^+ antiporter, were unchanged. A Δ*pacC* strain containing the $pacC^{CA}$ allele showed developmental defects including an increase in conidiation and formation of intrahyphal hyphae; phenotypes not observed in the wild type. Interestingly, *E. festucae* wild-type also hyperconidiates when grown at alkaline pH but not Δ*pacC*. In contrast to reports in other fungi, PacC is dispensable for growth of *E. festucae* at alkaline pH and does not appear to be required for secondary metabolite gene expression in either culture or *in planta*. *Lolium perenne* plants infected with Δ*pacC* had the same interaction phenotype as wild-type. However, plants infected with the constitutive active mutants (Δ*pacC*/$pacC^{CA}$) were hypertillered and the hyphae within the leaves formed aberrant structures indicating that PacC has an important but not essential role for establishment and maintenance of this symbiotic interaction.

456. The zinc transporter Znt1 regulates intracellular zinc homeostasis and influences virulence of the human fungal pathogen *Cryptococcus neoformans*. Eunsoo Do[1], Guanggan Hu[2], Mélissa Caza[2], James Kronstad[2], Won Hee Jung[1]. 1) Department of Systems Biotechnology, Chung-Ang University, Anseong, 456-756, Republic of Korea; 2) The Michael Smith Laboratories, Department of Microbiology and Immunology, University of British Columbia, Vancouver BC, V6T 1Z4, Canada.

Zinc is an essential nutrient in living organisms and a cofactor for various metalloproteins, including zinc finger DNA-binding proteins. To disseminate and survive, a pathogenic microbe must obtain zinc from the host, which is an environment with extremely limited zinc availability. In this study, we focused on defining the roles of putative zinc transporters Znt1 and Znt2 in the human pathogenic fungus *Cryptococcus neoformans*. Znt1 and Znt2 are homologous to Zrt1 and Zrt2 of the model fungus, *Saccharomyces cerevisiae*, respectively. We found that the expression of Znt1 was regulated by the zinc concentration in the environment and the zinc-responsive regulator Zap1. The mutant lacking *ZNT1* displayed a severe growth defect in zinc-limited conditions, while the mutant lacking *ZNT2* displayed normal

growth. ICP-AES analysis showed that the total cellular zinc levels were significantly decreased in the *znt1* mutant compared to the wild type, and overexpression of Znt1 was associated with increased cellular zinc levels, suggesting that Znt1 plays roles in zinc homeostasis in *C. neoformans*. We constructed a Znt1-Gfp fusion protein and found that the protein was localized in the vacuole. Furthermore, the mutant lacking *ZNT1* showed attenuated virulence in a mouse inhalation model of cryptococcosis. Overall, our data suggest that Znt1 plays essential roles in zinc homeostasis and in the virulence of *C. neoformans*.

457. Identifying the role of a *Cryptococcus* species-specific cell wall gene in response to the host. Shannon Esher[1], Maria Kohlbrenner[2], Kyla Ost[2], J. Andrew Alspaugh[2]. 1) Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC; 2) Department of Medicine, Duke University School of Medicine, Durham, NC.

Cryptococcus neoformans is an opportunistic fungal pathogen that causes life-threatening disease in immunocompromised hosts. The *C. neoformans* cell wall is a dynamic structure that this fungus carefully controls in response to its environment. Upon entering the host, *C. neoformans* dramatically alters its cell wall to facilitate immune avoidance and regulate the host-pathogen interface. We have identified *MAR1*, a novel *Cryptococcus* species-specific gene that is an important regulator of these host-induced cell wall changes. We have generated a *mar1Δ* mutant strain and shown that it has sensitivities to elevated temperature and alkaline pH, in addition to a capsule defect. Interestingly, this capsule defect is due to a defect in polysaccharide attachment to the cell wall, rather than polysaccharide biosynthesis. Using cell wall staining and biochemical assays, we have observed an increase in the immunogenic cell wall components, chitin and chitooligomers, specifically in host-mimicking conditions. By co-culturing *C. neoformans* with macrophages and assaying the induction of the inflammatory cytokine TNFα, we have shown that the *mar1Δ* mutant induces 4-5 times more TNFα production than wild type cells. These results suggest that Mar1 is regulating important cell wall changes in response to the host. In the absence of the Mar1 protein, the aberrant cell wall contains, and likely exposes, more chitin and chitooligomers, resulting in increased macrophage activation. Future studies will elucidate how Mar1 is regulating these cell wall changes, and what implications they have on the host immune response *in vivo*.

458. Screening mutants for enhanced lithium sensitivity identifies novel factors contributing to capsule elaboration and melanization in *Cryptococcus neoformans*. Francois Mayer, James Kronstad. Michael Smith Laboratories, Vancouver, BC, Canada.

Cryptococcus neoformans is one of the major human fungal pathogens and is estimated to account for more than half a million deaths annually. Despite being primarily found in the environment, this fungus can readily infect immunocompromised individuals such as HIV^+/AIDS patients. Three key virulence factors contribute to cryptococcal pathogenicity, including elaboration of a polysaccharide capsule, synthesis of melanin, and the capacity to grow at the physiological temperature of humans, i.e., 37°C. The cAMP-dependent protein kinase (PKA) signaling pathway mediates cellular responses including nutrient sensing, melanin synthesis and capsule production. Compromising PKA activity in *C. neoformans* results in decreased capsule elaboration and attenuated virulence. This phenotype is observed in a *pka1* mutant which lacks the catalytic subunit of PKA. In addition, a *pka1* mutant also displays a markedly enhanced sensitivity towards lithium chloride-induced stress. In this work we hypothesized that increased lithium sensitivity and defective capsule production may be linked. In order to identify novel factors contributing to capsule formation we screened 1,200 mutants for enhanced lithium chloride sensitivity. This initial screen identified approximately 100 mutants with such a phenotype. Selected mutants were re-investigated for their capacity to grow in the presence of elevated lithium concentrations on solid media. Eleven mutants with a reproducible lithium-sensitive phenotype were then analyzed for their capacity to generate capsule, produce melanin, and grow at 37°C. The exocyst component, Sec5, and the phospholipid methyltransferase, Opi3, were identified as novel factors contributing to capsule elaboration. Furthermore, Opi3, Sec5, the actin-associated protein, Rvs167, the Tor2-complex component, Avo1, and the predicted alginate lyase, CNAG_03413, were shown to be required for normal melanin production. Finally, Rvs167, Avo1, and Sec5 were demonstrated to contribute to normal growth at 37°C. This work illuminates the power of large scale screening approaches for the identification of novel potential virulence factors and sets the foundation for further in depth characterization of the above mentioned proteins.

459. Echinocandin Resistance in *Cryptococcus neoformans*: defining the roles of calcineurin-complex proteins. Kaila Pianalto[1], Andrew Alspaugh[1,2]. 1) Molecular Genetics and Microbiology, Duke University, Durham, NC; 2) Department of Medicine, Duke University, Durham, NC.

Echinocandins are the newest class of antifungal agents and are used extensively to treat infections due to several fungal pathogens. These drugs inhibit β-1,3-glucan synthase, a critical enzyme for cell wall biosynthesis. In *Cryptococcus neoformans*, caspofungin, one member of this drug family, is highly effective at inhibiting β-1,3-glucan synthase activity at the protein level; however, it is ineffective at inhibiting growth of *C. neoformans* in culture and *in vivo* at clinically relevant concentrations. The mechanism behind this paradoxical resistance is not well characterized. Here we have used a forward genetics approach to identify gene products in *C. neoformans* that mediate caspofungin resistance in this important fungal pathogen. In a preliminary screen, we have determined that a loss-of-function mutation in the gene encoding the calcineurin b regulatory subunit causes *C. neoformans* to become more susceptible to caspofungin. Mutation of the calcineurin a catalytic subunit confers a similar phenotype. Interestingly, by further characterizing other components of this pathway, we found that the major transcriptional regulator downstream of calcineurin in *C. neoformans*, Crz1, does not mediate this drug sensitivity phenotype, indicating that the calcineurin-mediated mechanisms of caspofungin resistance are not controlled by Crz1, but rather an alternative downstream component. Studies to identify Crz1-independent targets *of* calcineurin in *C. neoformans* will further assess the role that this protein complex plays in echinocandin resistance.

460. Identification of conserved and novel features of the alkaline response pathway in the fungal pathogen *Cryptococcus neoformans*. Kyla Selvig[1], Teresa O'Meara[2], Naureen Huda[1], Shannon Esher[1], J. Andrew Alspaugh[1]. 1) Department of Medicine, Duke University, Durham, NC; 2) Department of Molecular Genetics, University of Toronto, Toronto, CANADA.

The Rim/Pal pathway is a conserved fungal signaling pathway responsible for sensing and responding to alkaline extracellular pH. This pathway was first identified and characterized in members of the ascomycete phylum. Here, we analyze the Rim/Pal pathway in the

opportunistic fungal pathogen, *Cryptococcus neoformans*, a member of the basidiomycete phylum. We found that like in ascomycete fungi, the *C. neoformans* Rim101 transcription factor is proteolytically activated in response to alkaline pH. Rim101 activation requires the ESCRT pathway along with three conserved Rim pathway components, Rim23, Rim20, and Rim13. Interestingly, *C. neoformans* and related basidiomycete fungi lack clear orthologs for the most upstream components of the pathway, which are responsible for sensing extracellular pH and activating the pathway. To identify these unknown Rim signaling components, we performed a random mutagenesis screen specifically designed to identify activators of the *C. neoformans* Rim pathway. From this screen, we identified a novel protein, which we named *RRA1* (Required for Rim101 Activation 1). *RRA1* appears to be specifically conserved in basidiomycete fungi, indicating that *RRA1* is likely a previously unidentified component of the basidiomycete Rim pathway. Supporting this hypothesis, we found that disrupting the *RRA1* ortholog in *Cryptococcus gattii* produced mutant phenotypes identical to a *C. gattii rim101Δ* mutant. *RRA1* is predicted to encode a membrane protein that is structurally similar to the Rim21/PalH pH membrane receptor in the ascomycete Rim pathway. However unlike the Rim21/PalH pH receptor, Rra1 does not appear to be localized on the plasma membrane or on intracellular vesicles, but is primarily localized to the endoplasmic reticulum. Future experiments will determine the specific role of Rra1 in Rim pathway activation, and whether the ER is the true site of Rra1 function. These studies have significantly advanced our understanding of the mechanisms of environmental sensing and adaptation in *C. neoformans* and other basidiomycetes.

461. The SCF(Fbp1) E3 ligase is a key regulator of *Cryptococcus*-host interaction during lung infection. Tong-Bao Liu[1], Venessa Espinosa[2], Yina Wang[1], Amariliz Rivera[2], Chaoyang Xue[1,3]. 1) Public Health Research Institute; 2) Department of Medicine; 3) Department of Microbiology, Biochemistry, and Molecular Genetics, Rutgers university, Newark, NJ.

Cryptococcus neoformans is a facultative intracellular fungal pathogen that infects the lung and then often disseminates to the central nervous system to cause meningitis. Alveolar macrophages are the first line of host defense against *Cryptococcus* infection. How *Cryptococcus* is able to suppress host immunity and overcome the antifungal activity of macrophages is still not clear. We reported that the F-box protein Fbp1, a subunit of the SCF^{Fbp1} E3 ligase, promotes *Cryptococcus* virulence by regulating the intracellular proliferation inside macrophages. Importantly, the *fbp1Δ* mutant elicits superior protective Th1 host immunity in the lung as compared to the wild-type strain, suggesting that Fbp1 functions as part of a mechanism leading to immune suppression. Furthermore, the role of Fbp1 in fungal infection is independent of known virulence factors (capsule, melanin, and growth at 37°C), suggesting that the SCF^{Fbp1} E3 ligase regulates a novel virulence control mechanism. Besides its critical role in fungal virulence, Fbp1 also controls fungal sporulation. Because E3 ligases regulate cellular activities by controlling the turnover of their substrates, we sought to identify Fbp1 substrates required for Fbp1-mediated pathogenicity and sporulation by employing a combination of genetic and proteomics approaches. We discovered that inositol phosphosphingolipid phospholipase C1 (Isc1) is an Fbp1 substrate involved in *Cryptococcus* growth in macrophages. Consistent with the hypothesis that Fbp1 mediates the Isc1 degradation, overexpression of *ISC1* mimics *fbp1Δ* phenotype by triggering a higher Th1 response during lung infection. We are currently investigating the Fbp1-mediated regulation of sphingolipid biosynthesis and its role in *Cryptococcus* virulence. We also identified CDK-related kinase Crk1 as an Fbp1 substrate which regulates fungal sporulation. The long-term goal of this project is to identify and characterize Fbp1-regulated fungal effectors and corresponding host immune factors that determine disease progression, which may lead to future development of novel antifungal drug targets.

462. Assessing fungal diversity in northern tropical Australia through fruiting body surveys, DNA barcoding and MiSeq amplicon pyrosequencing. Gregory Bonito[1], Matthew Barrett[3], Frank Udovicic[2], Teresa Lebel[2]. 1) Michigan State University, East Lansing MI, USA; 2) Royal Botanic Gardens, Melbourne VIC, Australia; 3) King's Park and Botanic Garden, Perth WA, Australia.

Australia exhibits an impressive spectrum of fungal diversity and endemism. Active collecting in the top-end of Queensland and Western Australia over the past 5 years has led to the discovery of novel species and evidence of links between northern tropical and southern Australian macrofungi, but also to SE Asian lineages. While collections from southern Australia are fairly extensive, those from tropical Northern Territory Australia are sparse and remain poorly characterized. In order to assess fungal biodiversity in tropical NT we carried out a two-week collecting expedition within eucalypt woodland and rainforest habitats during the wet season to collect fungal fruiting bodies and soil samples for DNA-based fungal diversity assessments. In total, we made 424 collections of macrofungi from across 11 sites, nearly quadrupling the number of recorded collections from this region. DNA reference sequences (ITS & LSU rDNA barcodes) were generated for around 90% of these collections. We also generated MiSeq amplicon ITS and LSU rDNA sequence data from 28 soil samples taken from seven of the sites to assess soil fungal diversity. After quality filtering, removing singletons and picking ITS1 OTUs with Usearch, there were an average of 13,500 sequences per library and 82% of the estimated OTUs/site were observed. Fungal communities of soil samples taken from the same sites loosely clustered together. Ectomycorrhizal species, particularly those belonging to the Amanitaceae, Boletaceae, and Russulaceae, were common and diverse in both fruiting body and DNA-based soil assessments. Many of the dominant OTUs in soil samples mapped to reference DNA barcodes recovered from fruiting body collections, and these reference sequences proved to be particularly useful in applying taxonomy to our MiSeq OTUs. Additional details on species diversity and distribution patterns of northern tropical Australian fungi will be discussed.

463. Using metatranscriptomics to characterize functional shifts in endophytic fungi at plant senescence: Are endophytic fungi latent saprotrophs? Ko-Hsuan Chen[1], Hui-Ling Liao[1], A. Elizabeth Arnold[2], Francois Lutzoni[1]. 1) Biology, Duke University, Durham, NC; 2) School of Plant Sciences and Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, AZ.

Endophytic fungi live inside healthy plants but do not cause obvious symptoms. The same fungal species can often be isolated from living and senescent tissues of the same plant, consistent with the widespread hypothesis that many endophytic fungi are saprotrophs during part of their life cycle. However, these investigations are mostly based on culture-dependent or DNA-based environmental sampling methods. It is not known whether fungal strains detected in healthy and senescent plant tissues are functionally active under both conditions, and which genetic mechanisms are involved in this functional transition. To gain a better understanding of endophytism-saprotrophism transitions *in situ*, we sequenced the metatranscriptome of the broom moss *Dicranum scoparium*, and its microbiota, which

were sampled from local forest sites in North Carolina. Three distinct layers (healthy, senescent, and dead tissue) can be recognized from the top to the base of the gametophytes of *D. scoparium*. We extracted total RNA from these three layers. Illumina Hi-Seq sequencing generated about 39 million qualified reads for each individual layer sample. By using NCBI GenBank and a customized database of the DoB project (Dimensions of Biodiversity; EndoBiodiversity.org), we were able to extract reads that mapped to the D1/D2 region of fungal ribosomal large subunit gene using Bowtie2. Through *de novo* assembly using Velvet, followed by BLASTN, we identified several fungi that are functionally active throughout the three gametophytic layers of *D. scoparium*, including: *Rickenella*, *Sistotrem*a, *Cladophialophora*, *Epibryon* and *Mortierella*. We also found fungal taxa that were active only in the youngest, photosynthetic, layer (e.g., *Hyaloscypha*). We further compared gene expression patterns of fungi under various degrees of host senescence to determine the molecular mechanisms involved in transitions between saprotrophism and symbiotrophism. Our study sheds light on the potential for ecologically important functional shifts in host use.

464. Endohyphal bacteria of tropical Sordariomycetes: community structure and relationships with other functional groups of bacteria in a lowland tropical rainforest. Justin P. Shaffer[1], Rachel E. Gallery[2,3], David A. Baltrus[1], A. Elizabeth Arnold[1,3]. 1) School of Plant Sciences, The University of Arizona, Tucson, Arizona USA 85721; 2) School of Natural Resources and the Environment, The University of Arizona Tucson, Arizona USA 85721; 3) Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, Arizona USA 85721.

Bacterial endosymbionts that inhabit mycorrhizal Gigasporaceae, pathogenic Mucorales, and endophytic Dikarya can profoundly alter the outcomes of plant-fungus interactions. However, their presence, phylogenetic relationships, and ecological importance have not yet been examined systematically among the most species-rich clades of fungi, nor in some of the most hyperdiverse regions on Earth. Here, we report the phylogenetic relationships and community structure of bacterial endosymbionts (endohyphal bacteria, EHB) living within Sordariomycetes, one of the most diverse classes of Ascomycota, in a lowland tropical forest in Panama. We isolated fungi from surface-sterilized seeds of pioneer trees recovered following soil exposure, and from mature tissues of co-occurring woody plants. We used a PCR-based assay to detect bacteria, and confirmed their presence using fluorescence microscopy. Analyses highlight the phylogenetic relationships and diversity of EHB and their hosts, and compare EHB with seed-associated- and free-living soil-bacteria from the same host seeds and soil lots. Our results reveal that phylogenetically diverse bacteria are common among tropical plant-associated fungi representing highly diverse orders of Sordariomycetes. The phylogenetic diversity of tropical EHB exceeds that of comparably sampled bacterial communities found in tropical seeds and soil, or in studies of EHB from the temperate zone. The capacity to grow endohyphally does not exhibit significant phylogenetic signal, suggesting multiple origins across the bacterial tree of life. EHB from seed-associated fungi are similar in terms of community composition to bacteria living in seeds, suggesting an environmental source for bacteria that colonize fungal hyphae. Ongoing work will assess effects of these bacteria on fungal phenotypes and plant-fungus interactions.

465. Common molds modify plant disease. Posy Busby[1], Kabir Peay[2], George Newcombe[3]. 1) Biology, Duke University, Durham, NC; 2) Biology, Stanford University, Stanford CA; 3) College of Natural Resources, University of Idaho, Moscow ID.

Posy E. Busby[1,2,*], Kabir G. Peay[3], George Newcombe[2] , [1]Biology Department, Duke University, Durham, North Carolina 27708, [2]College of Natural Resources, University of Idaho, Moscow, Idaho 83844, [3]Department of Biology, Stanford University, Stanford California 94305

Microfungi regarded as common molds (e.g., *Alternaria*, *Cladosporium*) occur in plant leaves as non-pathogenic leaf endophytes. Some endophytes are known to decrease or increase disease severity in their host plants, serving as defense mutualists or pathogen enablers, respectively. However, the generality of endophytes in modifying leaf disease severity has been explored rarely, and has not been coupled with studies of their abundance and distribution in wild populations. We used next-generation DNA sequencing to characterize the fungal leaf microbiome of *Populus trichocarpa* in wild populations throughout the Pacific Northwest (USA) and to determine how the relative abundance of common leaf endophytes correlated with the severity of a major leaf rust disease of *P. trichocarpa*, *Melampsora*. We observed both positive and negative correlations between the relative abundance of common endophytes and rust severity. Using controlled inoculation experiment, we then confirmed that the endophytes modify rust disease severity in the predicted directions. Our results demonstrate that disease modification is a central function of common endophytes within the leaf microbiome of *P. trichocarpa* and that leaf endophytes help explain geographic variation in plant disease severity.

466. *Ralstonia solanacearum* lipopeptide induces chlamydospore formation followed by bacterial entry in close encounters with fungi. Joe Spraker[1], Laura Sanchez[2], Pieter Dorrestien[2], Nancy Keller[3]. 1) Plant Pathology, University of Wisconsin - Madison, WI; 2) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California- San Diego, CA; 3) Bacteriology, Medical Microbiology and Immunology, University of Wisconsin- Madison, WI.

The polymicrobial consortium within the rhizosphere communicates by chemical signaling that, ultimately, impacts survival in symbioses. Here we characterize the endosymbiotic interaction of two economically important plant pathogens, *Aspergillus flavus* and *Ralstonia solanacearum*. Using a variety of histological techniques we show that fungal chlamydospore-like structures form in response to a diffusible compound produced by *R. solanacearum.* Imaging Mass-Spec (IMS) and targeted genetic deletion show this metabolite to be a new lipopetide named ralsolamycin. Confocal scanning laser microscopy with a GFP *R. solanacearum* isolate show bacterial internal colonization of chylamydospores, indicating a newly described endofungal lifestyle for this important plant pathogen.

467. Tools for Functional Analysis of Effector Genes in *Phytophthora sojae*. Felipe Arredondo[1], Shiv Kale[2], Megan Asula[1], Tian Zhou[2], Hua Wise[1], Brett Tyler[1]. 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR 97333; 2) Bioinformatics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060.

Plants produce a molecular defense response when they are challenged by a plant pathogen. *Phytophthora sojae*, a soybean pathogen,

produces effector proteins to promote infection. Effectors include Avirulence (Avr) genes products that can interact with plant resistance proteins (Rps), if present, resulting in effector-trigger immunity. There are possibly hundreds of effector genes in *Phytophthora* species that are involved during infection of a plant host. Understanding the function of these genes during infection is still not clear and more studies need to be done in this complex system. Our lab uses several molecular tools to assess the role of these genes in pathogenicity. Some of these tools and methodology could be used in other *Phytophthora* species to confirm and support evidence of effector genes. An effective and reliable method is the protoplast/PEG transformation with DNA or dsRNA. These methods can introduce fluorescent proteins as cytological markers or over express or silence a target gene. Silencing genes via DNA or dsRNA can produce stable or transiently silenced transformants respectively. Silencing effector genes in *P. sojae* has been used to test the role of many effector genes during infection. Another molecular tool is the double-barrel particle bombardment, which enables transient expression of *P. sojae* genes in soybean tissue and accurate measurement of the effects of that expression.

468. Homologs of *Verticillium dahliae* effector Ave1 contribute to virulence in fungal pathogens of diverse plant hosts. Jordi C. Boshoven[1], Malaika Ebert[2], Melvin D. Bolton[2], Bart P.H.J. Thomma[1]. 1) Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, The Netherlands; 2) Agricultural Research Service, Northern Crop Science Laboratory, US Department of Agriculture, Fargo, ND 58102.

Verticillium dahliae is a fungal pathogen that causes vascular wilt in a broad range of host plants, including commercially important crops. The immune receptor Ve1, of which homologs are found in several host plants, confers resistance to *Verticillium* race 1 strains in tomato. Genome and RNA sequencing of *V. dahliae* race 1 and race 2 strains resulted in the identification of the highly expressed race 1-specific *Ave1* gene that encodes the effector protein that is recognized by Ve1. Deletion of *V. dahliae Ave1* does not only result in loss of recognition on *Ve1* plants, but also makes the fungus less aggressive on tomato plants lacking *Ve1*. Homologs of *Ave1* were mainly found in plants, but also in the fungal plant pathogens *Fusarium oxysporum, Cercospora beticola* and *Colletotrichum higginsianum.* To determine whether these *Ave1* homologs can contribute to virulence, *V. dahliae Ave1* deletion strains were complemented with the homologs of *F. oxysporum, C. beticola* and *C. higginsianum*, and tested for aggressiveness on tomato plants lacking *Ve1*. None of the tested homologs complemented virulence of *V. dahliae Ave1* deletion strains. Subsequently, *Ave1* deletion strains were generated in *F. oxysporum, C. beticola* and *C. higginsianum* to study their contribution to virulence in these pathogens. *Ave1* homologs found in these three pathogens contribute to virulence. These data may suggest that *Ave1* homologs of *V. dahliae* on the one hand, and *F. oxysporum, C. beticola* and *C. higginsianum* on the other hand, contribute to fungal virulence in different manners.

469. Understanding the *Parastagonospora nodorum* – wheat interaction; is it as simple as we think? Susan Breen, Britta Winterberg, Peter Solomon. Research School of Biology, The Australian National University, Canberra, ACT, Australia.

It has long been thought that necrotophic pathogenic fungi use lytic enzymes to break down plant cells to access nuritents held within. In recent years it has emerged that some necrotrophic fungi possess a more complicated and specific infection strategy, appearing reliant on a gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen *Parastagonospora nodorum*, it has been demonstrated that the basis of this host specific interaction is small cysteine-rich effector proteins scereted during infection (ToxA, Tox1 and Tox3). It is hypothesised that these effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, wilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood. As part of a larger project to elucidate the mechanisms of these necrotrophic effectors, we are searching for potential host protein binding partners for the Tox3 effector. In this work, the host target of Tox3, PR1-1 was identified through a Yeast Two Hybrid approach. Importantly, the interaction of Tox3 and PR1-1 was confirmed *in planta* using Co-IP. Recently, it was demonstrated that ToxA interacted with another PR1 protein closely related to PR1-1, PR1-5 (Lu et al., 2014). Our analysis confirmed that Tox3 was also able to bind to PR1-5 leading to the hypothesis that Tox3 targeted the basic group of PR1 proteins. We then assessed if Tox3 could interact with other classes of PR1 proteins. These analyses showed that Tox3 can interact with basic and acidic proteins but not with basic proteins with a CTE. Subsequent site-directed mutagenesis experiments have identified critical amino acids involved in this interaction for both Tox3 and the PR1 proteins. We have also used infiltration studies of heterlogously-expressed mutant Tox3 proteins to determine the requirement of its binding to PR1-1 to cause necrosis. These findings have raised the question as to the role of the PR1 proteins during infection and the consequence of it binding to the Tox3 effector. Is the binding of Tox3 to these PR1 proteins required for necrosis, or is it a mechanism to dampen host defence?

470. *F*usarium *R*apid *AL*kalinization *F*actor (*f-ralf*) encodes a secreted virulence effector acquired by horizontal gene transfer from plants. Sara Masachis[1], David Turrà[1], Mennat El Ghalid[1], Georg Felix[2], Thomas A. Richards[3], Antonio Di Pietro[1]. 1) Department of Genetics, University of Cordoba, Spain; 2) Center of Plant Molecular Biology, Eberhard-Karls-University Tübingen, Germany; 3) Biosciences, University of Exeter, UK.

Fungal pathogens secrete effector molecules to shape the host environment for their own benefit. For instance, extracellular alkalinization is used by a number of phytopathogens to improve colonization of the host tissue. We previously showed that in the root-infecting fungus *Fusarium oxysporum* an increase in extracellular pH promotes invasive growth and pathogenicity on tomato plants. The mechanisms that mediate extracellular alkalinization during fungal infection are poorly understood. Inspection of the *F. oxysporum* genome sequence identified a gene encoding a predicted secreted homolog of *R*apid *AL*kalinization *F*actor (RALF), a conserved family of plant peptide hormones that regulate cell expansion during plant growth and development. Expression of *ralf* is markedly upregulated in *F. oxysporum* during infection of tomato roots. Synthetic F-RALF peptide triggered rapid alkalinization in tomato cell cultures and caused inhibition of root elongation and root hair growth both in tomato and Arabidopsis plants. Fungal mutants lacking the *f-ralf* gene were significantly attenuated in virulence on tomato plants, and induced expression of defense genes in the host. Alkalinization of the extracellular medium was observed in tomato plants exposed to the *F. oxysporum* wild type and complemented strains, but not the *f-ralf* mutants. A survey of

sequenced fungal genomes identified *ralf* homologs in a number of phylogenetically distant species, all of them plant pathogens. We propose that these fungi use RALF peptides acquired by horizontal gene transfer from plants to enhance their infectious potential through alkalinization of the host tissue.

471. Effector-driven adaptation of *Aphanomyces* species to animal or plant hosts. Elodie Gaulin, Michiel JC Pel, Sarah Courbier, Laurent Camborde, Helene San Clemente, Bernard Dumas. LRSV, UMR5546 CNRS Univ P Sabatier, Castanet, France.

Oomycetes are fungal-like microorganisms related to chromophyte algae and other heterokont protists, including major plant and animal pathogens threatening agricultural and natural ecosystems. Within the phylum Oomycota, most of the plant pathogens belong to the Peronosporale lineage whereas the Saprolegniale lineage includes animal pathogens and also some phythopathogenic species notably in the genus *Aphanomyces*. This unique oomycete genus comprises highly specialised species pathogenic on plants, fishes and crustaceans. This diversity offers the opportunity to decipher mechanisms driving adaptation to unrelated hosts. To get insight in these mechanisms, we performed genome sequencing of 10 strains of *Aphanomyces* sp. representing the biological diversity of the genus. A combination of sequencing technologies led to the generation of a 61 Mb reference genome for an *A. euteiches* strain causing important damage on pea crops. Three genome sequences of strains of the crayfish pathogen *A. astaci* with low and high virulence on crayfishes have been obtained. A comparative genomics approach was developed to identify effector repertoires which could be involved in adaptation of *Aphanomyces* species to various hosts, by focusing on genes coding secreted proteins. This analysis revealed that animal and plant pathogenic species harbor specific effector repertoires which have been shaped by various evolutionary processes including expansion, gain or loss of gene families. Strikingly, plant pathogenic *Aphanomyces* species have expanded gene families coding plant-cell wall degrading enzymes, which are absent in animal pathogenic species, and acquired a specific family of small secreted proteins highly expressed during pathogenesis. In animal pathogens, expansion of secreted protease genes occurred and a lineage-specific gene family coding secreted proteins harboring a chitin-binding module putatively involved in degradation of crustacean shell has been identified. Together, these data allowed the identification of new classes of pathogenicity effectors acting on plant or animal hosts and give clues on evolutionary mechanisms shaping adaptation of pathogenic oomycetes to unrelated hosts.

472. Characterization of the novel *Cercospora beticola* necrosis-inducing effector *CbNIP10*. Malaika Ebert[1,2], Bart Thomma[2], Melvin Bolton[1]. 1) USDA - ARS, Fargo, ND, USA; 2) Laboratory of Phytopathology, Wageningen University, The Netherlands.

Cercospora Leaf Spot (CLS), caused by the hemibiotrophic fungus *Cercospora beticola*, is the most destructive foliar disease of sugar beet worldwide. During infection, plant pathogens secrete effectors that help establish disease. Although many effectors have been characterized in other pathosystems, no *C. beticola* effector proteins have been reported to date. To identify *C. beticola* proteins involved with virulence, we grew *C. beticola in vitro* under specific conditions and tested culture filtrate for necrosis-inducing activity by infiltration into sugar beet leaves. Culture filtrate from one growth condition reliably caused necrosis in sugar beet leaves within 24 h. Treatment of culture filtrate with a mixture of proteases abolished necrosis-inducing activity, confirming that the *C. beticola* effector(s) responsible for necrosis was proteinaceous in nature. Active culture filtrates were partially purified using liquid chromatography (LC). A single (LC) fraction was repeatedly identified that caused necrosis upon infiltration into host tissue. MS/MS analysis of this fraction identified three *C. beticola* proteins. Each protein exhibited classic effector characteristics, including secretion signal, high cysteine content and low molecular weight (6 to 11 kDa). Candidate effector proteins were produced in *Pichia pastoris*. Infiltration of the candidate effector CbNIP10 caused necrosis in sugar beet leaves, while the other two effector candidates did not. A detailed characterization of CbNIP10 will be presented. Gaining an understanding of *C. beticola* effector biology will give new insight in *C. beticola* pathology and may lead to novel CLS control measures.

473. Interference of oomycete effectors with plant development. Edouard Evangelisti, Ayoub Kadoussi, Mehdi Doumane, Sebastian Schornack. Sainsbury Laboratory Cambridge University (SLCU), Cambridge, United Kingdom.

To be successful in conquering the host, microbes exploit effector proteins to target plant immunity. Beside immune-suppressing activities, recent reports suggest that plant pathogens also secrete proteins which interfere with other general plant processes, such as development and hormone physiology. In this study, we identified a subset of secreted RXLR-EER proteins from the oomycete *Phytophthora palmivora* which when expressed in planta interfere with leaf, flower or trichome development in *Arabidopsis thaliana* and *Nicotiana benthamiana*. Further work suggests that these proteins target processes not previously implied in Phytophthora plant interactions and with no direct link to plant immunity. As such, they offer novel opportunities to understand oomycete infection strategies. Furthermore, they represent promising tools to study development processes complementary to often lethal whole-pant knockouts or knockdowns.

474. SnTox1, a *Parastagonospora nodorum* necrotrophic effector, elicits PCD while binding chitin to protect the pathogen from wheat chitinases. T.L. Friesen[1], Z.H. Liu[2], Y.M. Kim[2], Y. Gao[2], P.J.G.M. de Wit[3], J.D. Faris[1]. 1) Cereal Crops Res Unit, USDA-ARS, Fargo, ND; 2) Department of Plant Pathology, North Dakota State University, Fargo ND; 3) Wageningen University, Wageningen, The Netherlands.

Parastagonospora nodorum (Synonym: *Stagonospora nodorum*) is a destructive pathogen of wheat that induces yield and quality losses by causing necrosis on the leaves and glumes of wheat. *P. nodorum* is a necrotrophic specialist pathogen that secretes an arsenal of necrotrophic effectors (NEs) involved in inducing necrosis. SnTox1 was the first of seven NEs to be reported from *P. nodorum* and interacts directly or indirectly with the single dominant susceptibility gene *Snn1*. SnTox1 is recognized by *Snn1* followed by the induction of a classical defense response involving programmed cell death (PCD), an oxidative burst, DNA laddering, and up regulation of several PR proteins including chitinases. However, this recognition results in wheat being susceptible to *P. nodorum* rather than resistant. Interestingly, the C-terminus of SnTox1 has homology to several plant chitin binding proteins and we have shown that SnTox1 does bind chitin. Therefore, we have expressed two wheat chitinases that are able to degrade the fungal cell wall in vitro to show that *P. nodorum*

strains harboring *SnTox1* are significantly more protected from chitinase degradation than the isolates without *SnTox1*. We also transformed other plant pathogens and non-pathogens with *SnTox1* including *Neurospora crassa* (saprotroph), *Pyrenophora teres* f. *teres* (necrotrophic barley pathogen), and *Cercospora beticola* (hemi-biotrophic sugarbeet pathogen) and show that not only did some of these fungi become pathogens of wheat lines harboring *Snn1*, but all had increased protection from the wheat chitinases in vitro, exposing a secondary or possibly the primary function of SnTox1. The dual function of this protein explains the high prevalence of *SnTox1*, relative to other NEs in the *P. nodorum* global population and shows that necrotrophs harbor chitin binding proteins for their protection during pathogenesis.

475. Effectors from the plant pathogenic *Aphanomyces euteiches* trigger host DNA damage. Elodie Gaulin, Michiel JC Pel, Laurent Camborde, Diana Ramirez, Hélène San-Clemente, Bernard Dumas. UMR5546 CNRS-Université , Laboratoire de Recherche en Sciences Végétales, Castanet-Tolosan, France.

Microbial pathogens translocate effectors inside host cells to subvert cellular functions and suppress immune responses. Oomycetes, which are fungal-like eukaryotic microorganisms that cause some of the most destructive plant diseases in the world, secrete several different kind of effector proteins. Two large groups of these effectors are the RXLR and the CRN (Crinkler) proteins. RXLRs and CRNs are modular proteins with conserved N-termini and highly diverse C-terminal effector domains. We recently obtained the genome sequence of the legume root pathogen *Aphanomyces euteiches (ATCC201684, AphanoDBv2.0; https://www.polebio.lrsv.ups-tlse.fr/aphanoDB/)*. This data revealed the absence of RXLR effectors and the presence of over 150 putative CRN effectors in the genome of this pathogen. *Aphanomyces* sp. CRNs are characterized by the presence of an LYLALK translocation motif, and although many CRNs have been identified data on CRN function and targets is still limited. We started the functional analysis of these CRN effectors to gain insights in the virulence mechanisms of *A. euteiches* and to identify possible targets for disease control. We have been able to show that one of the CRN effectors, CRN13, localizes in the plant nucleus where it triggers cell death. Further, we found that the CRN13 ortholog of the fungal amphibian pathogen *Batrachochytrium dendrobaditis* is able to cause a similar response in both plant and amphibian cells. Additionally, we demonstrated that both CRN13s are able to bind DNA *in vitro* and cause DNA damage *in vivo*. Altogether, this work reveals that CRN effectors produced by unrelated plant and animal pathogens bind DNA to interfere with host cell development.

476. R1-mediated immunity triggered by the *Phytophthora infestans* RXLR effector AVR1 is activated inside the nucleus. Yu Du, Klaas Bouwmeester, Jeroen Berg, Francine Govers. Lab. of Phytopathology, Wageningen University, Wageningen, Netherlands.

Phytophthora infestans is a devastating plant pathogen that causes late blight on potato and tomato. To colonize host plants, *P. infestans* secretes effectors that can modulate host defence. Well-known are the RXLR effectors that are translocated into host cells to manipulate the cell machinery. To counteract the pathogen, potato exploits nucleotide-binding leucine-rich repeat (NLR) immune receptors that confer resistance against *P. infestans* upon recognition of a RXLR effector, with each NLR protein (or R protein) having its own matching RXLR effector (or AVR protein). The mechanisms underlying NLR-mediated resistance are still poorly understood. In this study we exploited fluorescent tags and nuclear localization and export signals (NLS/NES) for determining the subcellular localization of the potato NLR protein R1 and the *P. infestans* RXLR effector AVR1, and for targeting these proteins to nucleus or cytoplasm. Microscopic imaging revealed that both R1 and AVR1 occur in nucleus and cytoplasm, and in close proximity. Transient expression of NLS- or NES-tagged R1 and AVR1 in *Nicotiana benthamiana* showed that activation of R1-mediated hypersensitive response and resistance requires localization of the R1/AVR1 pair in the nucleus. However, AVR1-mediated suppression of cell death in absence of R1 is dependent on localization of AVR1 in the cytoplasm. A balanced nucleocytoplasmic partitioning of AVR1 seems to be a prerequisite.

477. Prediction of SSPs involved in *Suillus* mutualism interactions. Hui-Ling Liao, George Greene. Duke University Durham, NC.

Ectomycorrhizal fungi (EMF) play crucial roles in plant function in ecosystems. The molecular bases underlying the symbiotic establishment of EMF remain largely unknown. Several recent studies have discovered the involvement small-secreted proteins in the EMF symbiosis and host recognition, including *Suillus* spp., the keystone member of pine associated EMF. Several species of *Suillus* exhibit host-specific associations with different *Pinus* spp. In this study, we used *Suillus* as a model system to identify the potential small-secreted proteins that are involved in symbiosis. We used genome-wide expression analysis to compare compatible *Suillus-Pinus* roots to the incompatible *Suillus-Pinus* root and *Suillus* culture samples across four species of *Suillus* and three species of *Pinus*. In general, over 12,000 abundantly expressed *Suillus* genes were detected in response to compatibility. An improved computational workflow was further applied to identify the small proteins that were smaller than 300 amino acids. Small proteins were considered secreted if they encoded signal peptides without any ER or Golgi retention signal motifs as well as transmembrane domains. Putative SSPs were characterized based on motif composition. Our results showed that Y/W/XC motifs as well as Cys-enrichment where highly represented in putative SSPs; however, no significant difference in motif composition is detectable when compared to the entire transcriptome. Our study on determining the potential effector-like proteins involved in symbiosis will provide insight into how the relationship is established and maintained in nature.

478. Enhanced resistance in *Theobroma cacao* against oomycete and fungal pathogens by secretion of phosphatidylinositol-3-phosphate-binding proteins. Emily Helliwell[1,2], Julio Vega-Arreguin[3], Zi Shi[2], Bryan Bailey[4], Shunyuan Xiao[5], Siela N. Maximova[2], Brett M. Tyler[1,2], Mark J. Guiltinan[2]. 1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Department of Plant Science and Huck Institute of Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA; 3) Virginia Bioinformatics Institute and Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA; 4) United States Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, USA; 5) Institute for Bioscience and Biotechnology Research & Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA.

Internalization of oomycete and fungal pathogen effectors into host plant cells has been reported to be blocked by proteins that bind to the

effectors' cell entry receptor, phosphatidylinositol-3-phosphate (PI3P). This finding suggests a novel strategy for disease control by engineering plants to secrete PI3P-binding proteins. We tested this strategy using the chocolate tree, *Theobroma cacao*, as a proof-of-concept crop system. Both transient and stable expression of secreted, functional PI3P-binding proteins in detached leaves of *T. cacao* greatly reduced infection by two oomycete pathogens, *Phytophthora capsici* and *Phytophthora palmivora* and the fungal pathogen *Colletotrichum theobromicola*. Microarray analyses revealed that cacao leaves transiently expressing a functional, secreted PI3P-binding protein showed a highly similar profile of differentially-regulated genes to control leaves infected with *P. capsici*. Many of the up-regulated genes were related to production of reactive oxygen intermediates, suggesting a form of priming response may be present. Staining with 3,3-diaminobenzidine (DAB) showed an increased basal level of hydrogen peroxide in cacao leaves with both stable and transient expression of functional PI3P-binding proteins. These results suggest that secretion of PI3P-binding proteins enhances resistance to oomycete and fungal pathogens, potentially by activating defense signaling in addition to the possibility of inhibiting effector entry.

479. A game of hide and seek: Analysis of the interaction between the avirulence genes *AvrLm3* and *AvrLm7* of *Leptosphaeria maculans*. Clemence Plissonneau, Marie-Helene Balesdent, Isabelle Fudal, Thierry Rouxel. BIOGER CPP, INRA, Thiverval Grignon, France.

Leptosphaeria maculans is an ascomycete responsible for the phoma stem canker, a damaging disease of *Brassica napus*. The deployment of cultivars harboring *Rlm* major resistance genes is the most effective way to control this disease. We recently identified a negative correlation between resistance phenotypes induced by avirulence genes *AvrLm3* and *AvrLm7* : when an isolate possesses a functional allele of *AvrLm7*, the *Rlm3* resistance induced by *AvrLm3* is hidden. Massive deployment of cultivars with *Rlm7* resistance gene in the fields currently occurs and as a consequence virulent isolates against *Rlm7* are in the process of emerging. *Rlm3*, which was previously ineffective but present in many cultivars, is becoming effective again. Such a negative interaction between two avirulence genes has only been identified in one other ascomycete, *Fusarium oxysporum* and offers opportunities for the sustainable management of specific resistances.

Following the cloning of *AvrLm7*, we combined several approaches (RNA-seq, *de novo* sequencing of an avirulent isolate, and eventually BAC clones sequencing) to clone *AvrLm3*. The gene sequence was located in a contig absent from the assemblies of the reference sequence genome of *L. maculans*. Like *AvrLm7*, *AvrLm3* encodes for a small secreted protein, rich in cystein residues and highly expressed at early infection stage. The genotyping of field isolates showed that *AvrLm3* is highly conserved in field populations, with no isolates displaying deletion of *AvrLm3*. Only one avirulent allele was identified and several alleles were identified in virulent isolates.

A very low number of isolates virulent towards both *Rlm3* and *Rlm7* has been identified. RNAi silencing of virulent and avirulent isolates has been undertaken to evaluate whether this unusual conservation is due to the importance of *AvrLm3* for fungal fitness.

480. Characterisation of *Ave1* orthologs in *Venturia* scab pathogens. Janet Wheeler[1], Patrick Kastner[1], Adam Taranto[1], Jason Shiller[1], Jordi Boshoven[2], Carl Mesarich[2], Bart Thomma[2], Cecilia Deng[3], Joanna Bowen[3], Kim Plummer[1]. 1) Dept Botany, La Trobe Univ, Melbourne, Victoria, Australia; 2) Wageningen University, The Netherlands; 3) Plant & Food Research Ltd, Auckland, New Zealand.

Most fungal effectors are genus, species or race-specific, however a few are more broadly conserved (e.g. ECP6), and some are discontinuously distributed within the Fungi (Ave1 & AvrLm6). Single orthologs of Ave1 from *Verticillium dahliae*, a virulence effector that activates *Ve1*-mediated resistance in tomato, have been identified in unrelated fungi (*Colletotrichum higginsianum, Cercospora beticola, Fusarium oxysporum* f. sp. *lycopersici*). A subset of these activate *Ve1*-mediated resistance in tomato (de Jonge et al. 2012). *Ave1* also shares similarity to an ortholog in the phytopathogenic bacterium *Xanthomonas axonopodis* pv. *citri*, as well as to a common family of plant natriuretic peptides and expansins, involved in plant homeostasis and plant cell wall modifications (de Jonge et al. 2012). We have identified highly expanded *Ave1*-like gene families (with conserved predicted cysteine patterns & 37-57% overall aa identity) in the biotrophic scab fungi, *Venturia inaequalis* (5 genomes) & *V. pirina*. The orthologs are closely associated with repeats in *Venturia* genomes, however only a few appear to be impacted by RIP. Like *VdAve1*, *Venturia* orthologs have a conserved intron in the 5'UTR, which causes problems for automated gene calling, especially those packages informed by transcriptome data. Several of the *Venturia* orthologs are up-regulated during leaf infection (RNAseq data), and some are also highly expressed during *in vitro* growth on cellophane (RNAseq and proteomic data, Cook et al. 2014). Synthetic peptides (36 & 39 amino acids) from two *V. inaequalis* Ave1 orthologs, based on conserved homeostasis-regulating domains of plant proteins, affected *Arabidopsis thaliana* protoplasts (swelling) and guard cells (collapse) of *Tradescantia* leaves in epidermal peels exposed to the peptides (0.1µM) in solution. We hypothesize that *Venturia* Ave1 proteins may play a role during biotrophic infection in disturbing plant homeostasis and promoting nutrient release from plant cells.

481. Characterization of necrotrophic effectors in the conifer pathogen *Heterobasidion annosum* s.l. T. Raffaello, F. O. Asiegbu. Department of Forest Sciences, University of Helsinki, Helsinki, Finland.

The basidiomycete *Heterobasidion annosum* s.l. is a filamentous fungus which is considered to be one of the most destructive conifer pathogen in temperate forests of the northern hemisphere. *H. annosum* s.l. is a species complex which comprises 5 main species: two north American types, *H. irregulare* and *H. occidentale*, and three European types, *H. annosum* s.s., *H. abietinum*, and *H. parviporum*. *Heterobasidion* is characterized by a dual life style; a saprotrophic stage characterized by growth and survival on dead wood and a necrotrophic stage during which the fungus actively kills the plant cell and feeds on the dead plant material. In this study we searched the *Heterobasidion* genome to retrieve putative fungal effectors capable of promoting the necrotrophic growth during the tree infection stage. The genes were selected based on the presence of signal peptide and disulfide bridges which are supposed to stabilize the protein folding, and the absence of transmembrane domains, mitochondrial, and nuclear localization. The final list comprises 58 putative necrotrophic

effectors with no predicted protein domains. The putative effectors were custom synthesized, cloned into pICH86988 plasmid using a golden gate approach and expressed in Nicotiana benthamiana by *Agrobacterium* mediated transformation. Given the absence of an established protocol to validate *Heterobasidion* effectors in the native pine trees, *Nicotiana benthamiana* was used as a model organism to investigate the function of the selected genes. The initial transcriptome results based on microarray analysis of *Heterobasidion* grown necrotrophically in pine inner bark (phloem) and sapwood (xylem) revealed that all the selected putative effectors were differentially expressed in these conditions. RT-PCR analyses and expression in Tobacco will further validate and facilitate to ascribe function to each of the effector candidates.

482. Characterization of genes encoding potential effector proteins from *Trichoderma* spp. and their role in the interaction with *Arabidopsis thaliana*. Claudia Adriana Ramirez Valdespino[1], Ma. Daniela Porras Troncoso[1], Alfredo Herrera Estrella[2], Vianey Olmedo Monfil[1]. 1) Biology Dept, University of Guanajuato. Guanajuato, Guanajuato, Mexico; 2) CINVESTAV-Langebio, Irapuato. Irapuato, Guanajuato, Mexico.

Trichoderma species are common soil inhabitants that are used as biocontrol agents against phytopathogens. *Trichoderma* has the ability to activate both induced systemic resistance (ISR) and systemic acquired resistance (SAR) in plants through MAMP's (Microbe-Associated Molecular Patterns), some of which are effector-like proteins capable of altering host-cell structure and function. These alterations trigger defense responses, such as the production of avirulence factors and elicitors. There are a limited number of studies on the role of these proteins in the establishment of beneficial interactions. The production of molecules with effector characteristics by *Trichoderma* has been reported, and some of them are classified as members of the cellulases, xylanases, expansin-like proteins and cerato-platanins families. These effectors have been implicated in the establishment of the plant-fungus interactions, activating SAR and/or ISR mechanisms in different plants. Currently, our group is searching for novel effector-like proteins in *Trichoderma* species active in the interaction with *A. thaliana*. Using bioinformatic tools, we selected 21 genes coding for possible effector-like proteins and determined their differential expression during the interaction with the plant. By q-PCR assays we confirmed that three genes are up regulated and one down regulated. To determine the factors involved in the induction and regulation of these effector candidates *in planta*, we selected two of them and analyzed their expression in different carbon and nitrogen sources. Our results revealed that none is subject to nitrogen or glucose catabolite repression. We generated *Trichoderma* null mutants of the four genes and an overexpressing strain for the down regulated one. Currently, we are evaluating their participation during the *Trichoderma-Arabidopsis* beneficial interaction as well as their antagonistic activity against phytopathogenic fungi.

483. Towards deciphering the functional role of arbuscular mycorrhizal effectors in the symbiosis. Natalia Requena, Ruben Betz, Meike Hartmann, Sven Heidt, Leonie Hacker. Molecular Phytopathology, Karlsruhe Institute of Technology, Karlsruhe, Germany.

Arbuscular mycorrhizal (AM) fungi colonize plant roots and provide plants with mineral nutrients, prominently phosphate, in exchange for carbohydrates. The mutualistic symbiosis is one of the most widespread and ancient on the earth. The wide host range of AM fungi and the conserved key features of the association with all involved plants (arbuscule formation, modulation of phosphate homeostasis and reprogramming of carbon partitioning) suggests that identical molecular mechanisms underlie the functioning of the symbiosis. With our discovery of the first AM fungal effector protein SP7 (Kloppholz et al., 2011) the paradigm that AM fungi are naïve colonizers of plants and that the symbiosis is exclusively controlled by the plant was challenged. We showed that SP7 modulates the MAMP triggered immunity of the plant and thus promotes biotrophy. This suggests that in all other AM symbioses, involving other AM fungal species and other plants, similar or identical mechanisms might operate. In addition, we predict that other effectors controlling additional symbiotic features must exist. With the recent outcome of the first AM fungal genome (Tisserant et al., 2013; Lin et al., 2014), novel effector candidates have been identified and their functional characterization is in progress. We have focussed our efforts towards effectors containing nuclear localization domains, including two prominent effector families, the SP7-like family and the Crinkler family, and a couple of few other proteins. The existence of the Crinkler effector family in AM fungi is interesting *per se*, as this effector family is only found in Oomycetes and Chytridiomycetes. Despite their name and the phenotype some of them induce when expressed *in planta* (crinkled leaves), some Crinkler effectors have been shown to supress cell death. The SP7-like family and the Crinkler family have share in addition the ability to enter the plant cell on their own. And while a putative entry motif has been ascribed for Crinkler proteins, the mechanism by which SP7-like proteins enter the plant cell is yet unknown. Here we will present our latest results concerning the functional characterization of AM fungal effectors and their role in symbiosis.

484. Fungal LysM effectors: more than pathogen tools for host modulation? Eduardo Rojas Padilla, Anja Kombrink, Andrea Sánchez-Vallet, Bart Thomma. Department of Phytopathology, Wageningen University, Wageningen, Netherlands.

Filamentous fungal effector biology has been studied as a mechanism to deregulate the immune response during colonisation of a host. During plant colonisation, LysM effectors like Mg3LysM from the wheat pathogen *Mycosphaerella graminicola* or Ecp6 from the tomato pathogen *Cladosporium fulvum* have been shown to block Chitin Triggered Immunity (CTI) through different strategies: while Mg3LysM binds chitin and protects the cell wall against chitinases, Ecp6 sequesters chitin fragments with ultrahigh affinity to avoid recognition. In nature, nonetheless, filamentous fungi also interact with a complex community of microorganisms inside and outside their host. Recent data revealed that the fungal LysM effector Mg1LysM from *M. graminicola* not also binds chitin monomers but it can bind N-Acetylmuramic acid as well, a bacterial cell wall component. In tests *in vitro*, MgLysMs have the capacity to prevent bacterial attachment to the hyphae, while conferring protection to the cell wall towards chitinases. Additionally, Mg1LysM protein prevents degradation of the hyphae in presence of antagonistic bacteria *in vitro*. This data suggest that LysM effectors may play a role in interaction with (antagonistic) bacteria protecting the cell wall against chitinases secreted by antagonist microbes or by scavenging chitin to avoid attraction of antagonistic microorganisms. Considering that around 300 LysM effector proteins have been predicted in more than 70 fungal species with different lifestyles including pathogens, endophytes, and saprophytes; it is interesting to explore what functions LysM effector proteins may have during interactions with other microorganisms in different environments.

485. Towards the determination of exact interaction mechanisms between the tomato immune receptor Ve1 and the fungal effector Ave1. H. Rovenich, Z. Zhang, Y. Song, B.P.H.J. Thomma. Lab of Phytopathology, Wageningen University, Wageningen, Gelderland, Netherlands.

Recognition of pathogen effectors, small secreted molecules that facilitate host colonization, represents the basis for plant resistance against well-adapted pathogens. In tomato, the *Ve1* gene confers resistance against race 1 strains of the vascular wilt pathogen *V. dahliae*. *Ve1* encodes a surface-localized leucine-rich repeat (LRR) receptor-like protein (RLP), which recognizes the recently identified secreted *V. dahliae* effector Ave1. Immunopurification of affinity-tagged Ave1 transiently co-expressed with HA-tagged Ve1 in tobacco showed that the receptor protein co-purifies with Ave1, indicating an interaction between the pathogen effector and the immune receptor. A mutational screen of extracellular solvent-exposed residues across the LRR domains within the C1 region of Ve1 showed that stable Ve1 mutant alleles of the two consecutive LRR regions LRR3-LRR8 and LRR20-LRR23 were compromised in their HR-inducing activity when co-expressed with Ave1. Accordingly, when challenged with race1 *V. dahliae*, transgenic *Arabidopsis* plants carrying the non-functional *Ve1* alleles displayed symptoms similar to inoculated non-transgenic controls. These results suggest that Ve1 functionality is determined by two distinct clusters of LRR domains that may be involved Ave1 perception. Despite the fact that further mutational analyses in combination with biochemical methodologies can be employed to further elucidate the interaction between the receptor and the effector proteins, structural information will greatly enhance our ability to improve those strategies.

486. *Zymoseptoria tritici* LysM effectors protect cell wall chitin against host chitinases. Andrea Sánchez-Vallet[1], Dirk-Jan Valkenburg[2], Raspudin Saleem-Batcha[3], Jeroen Mesters[3], Bruce McDonald[1], Bart PHJ Thomma[2]. 1) Plant Pathology, ETHZ, Zurich, Switzerland; 2) Phytopathology, Wageningen University, The Netherlands; 3) Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lubeck, Germany.

Zymoseptoria tritici (syn. *Mycosphaerella graminicola*) causes Septoria tritici blotch, a major disease on wheat. Understanding the mechanisms by which this filamentous fungus colonizes wheat is therefore highly relevant. Two main phases can be discriminated in the infection by this pathogen. In the first phase, *Z. tritici* grows slowly and without developing macroscopic symptoms. Immediately after the first necrotic spots appear, fungal growth spikes and pycnidia begin to form pycnidia which are essential for epidemic development. Although the fungus grows slowly in the first phase, it still needs to protect itself against the resistance responses of the plant. In order to achieve this, *Z. tritici* produces two LysM effectors, Mg1LysM and Mg3LysM. Mg3LysM is an essential virulence factor that prevents the activation of chitin-triggered immunity, as shown for its homologs in *C. fulvum* and *M. oryzae*. Both *Z. tritici* LysM effectors have the additional function of protecting fungal cell walls from host chitinases, which would otherwise inhibit fungal growth. LysM effectors are small secreted proteins which contain at least one LysM domain and bind chitin, but it remains to be determined how they exert their biological function. Mg1LysM and Mg3LysM may directly inhibit the function of chitinases or may bind to the cell wall to prevent access of chitinases. The high-resolution crystal structure of Mg1LysM revealed its capacity to oligomerize in the presence of chitin, indicating that it may wrap around the fungal cell wall and thus prevent chitinases from gaining access to the cell wall chitin.

487. Structure-function analysis of the fungal Avr4 core effector family. Amanda Kohler[1], Anthony Salvucci[1], Li-Hung Cheng[1], Nicholas Hurlburt[2], Benjamin Schwessinger[1], Andrew Fisher[2,3], Ioannis Stergiopoulos[1]. 1) Plant Pathology, UC Davis, Davis, CA 95616, USA; 2) Chemistry, UC Davis, Davis, CA 95616, USA; 3) Molecular and Cellular Biology, UC Davis, Davis, CA 95616, USA.

Chitin is a key structural polysaccharide of fungal cell walls and a potent inducer of innate immunity in plants and mammals. Consequently, fungi have evolved intricate mechanisms to guard chitin from the host immune system, including shielding it under a layer of surface glucans, enzymatically converting it to chitosan, and secreting chitin-binding lectins such as CfAvr4, an extracellular effector protein from the tomato pathogen *Cladosporium fulvum* that binds and protects fungal chitin from host-derived chitinases during infection. We have previously reported that functional orthologs of CfAvr4 are present in Dothideomycete fungi, suggesting that Avr4 is a “core” effector with a conserved biochemical function and role in virulence. Despite their low sequence homology the majority of Avr4 orthologs are still perceived by Cf-4, a trans-membrane receptor-like protein (RLP) from tomato that recognizes CfAvr4 and mediates resistance. How immune receptors such as Cf-4 are able to recognize so diverse in sequence pathogen effectors remains unknown. To obtain a mechanistic insight into Avr4 function and perception by Cf-4, we solved the 1.6Å resolution X-ray structure and further functionally characterized PfAvr4, an effector protein from the tomato pathogen *Pseudocercospora fuligena*. PfAvr4 shares 48% similarity to CfAvr4, while its tertiary structure reveals structural homology to tachycitin, a CBM14 lectin from the horseshoe crab *Tachypleus tridentatus*. Biochemical analyses indicate that PfAvr4 and CfAvr4 bind specificity to chitotriose, while binding to higher molecular weight chitin is facilitated by co-operative protein-protein interactions, to create a protective layer around chitin in cell walls that prevents host chitinases from accessing it. Functional analysis by site-directed mutagenesis revealed key amino-acids involved in chitin-binding and further established that the ability to bind chitin is independent of PfAvr4's ability to elicit HR in Cf4-tomato. Overall, the data presented provide an understanding of the structure-function relationships in the Avr4 core effector family.

488. Identifying essential effectors from the soybean pathogen *Phytophthora sojae*. Hua Wise[1], Ryan Anderson[2], John McDowell[2], Brett Tyler[1]. 1) Center for Genome Research and Biocomputing, Department of Botany and Plant Pathology, Oregon State University, and Virginia Bioinformatics Institute, Virginia Tech; 2) 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 to 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, PsAvh16, PsAvh180 and PsAvh240 showed substantially reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. These three effectors are being used as candidates in a high throughput screen system utilizing *Pseudomonas* Type III secretion system to screen for new resistance genes against *P. sojae*.

489. Investigating the Sporulation Pattern of *Zymoseptoria tritici,* a Pathogen of Wheat. Anna Tiley, Gary Foster, Andy Bailey. The School of Biological Sciences, The University of Bristol, United Kingdom.

Zymoseptoria tritici is an ascomycete fungus which causes Septoria Tritici Blotch, a major disease of wheat. A potential way to control this pathogen is to prevent asexual sporulation by inhibiting development of the asexual fruiting body and spores. The purpose of this research is to identify key genes involved in regulating this process by creating knock-out mutants impaired in asexual sporulation.

The recent sequencing of the *Z. tritici* genome has provided useful insights into the pathogen, and has been used to identify candidate genes for investigation. Comparative genomic analyses were firstly used to identify potential genes involved in asexual sporulation in *Z. tritici.* A total of 84 key genes already known to be important for this process in other model ascomycete fungi were BLAST searched against the *Z. tritici* database to find potential homologues. Molecular genetic analyses were then conducted on 21 candidate genes to elucidate their temporal and spatial expression patterns across the time course of infection. From the genes analysed, 7 were selected for knock-out experiments in *Z. tritici.*

The knock-out plasmids containing a Hygromycin resistance cassette have since been constructed using homologous yeast recombination, and inserted into *Z. tritici* via *Agrobacterium tumefaciens* mediated transformation. The phenotypes of these mutants are currently being characterised and assessed for their ability to sporulate. Reliable sporulation of *Z. tritici* has also been successfully established *in vitro.* This will help future research into the fungus by allowing the uncoupling of sporulation from virulence.

490. Identification of a novel antibacterial protein from *Lentinula edodes*. N. van der Velden, N. Kaelin, M. Kuenzler, M. Aebi. ETH Zuerich, Switzerland.

Fungi produce a wide variety of toxic compounds in order to survive in their natural environment. These toxins can range from secondary metabolites and peptides to proteins. Most antibacterial compounds from fungi have been isolated from ascomycetes and are mainly secondary metabolites. The occurrence of antibacterial compounds from basidiomycetes is much less documented although it is known that fruiting body extracts of many basidiomycetes show antibacterial activity. In our laboratory, we recently identified and characterized a novel defensin, secreted by the vegetative mycelium of the model basidiomycete *Coprinopsis cinerea,* based on its antibacterial activity against *Bacillus subtilis* (Essig et al., 2014).

For the identification of novel antibacterial compounds, fruiting body extracts of several basidiomycetes were tested for their activity against *B. subtilis* and *Escherichia coli*. The extract of *Lentinula edodes* showed strong activity against *B. subtilis*. The activity was still present after dialysis and abolished after heat treatment. These results indicate that the antibacterial activity is protein based. Further experiments focus on identifying and purifying this antibacterial protein.

491. Gene expression profiling of the *Fusarium graminearum*-corn interaction during stalk rot disease. Liliana M. Cano[1], Jameel M. Al-Haddad[2], Frances Trail[2], C. Robin Buell[2], Lina M. Quesada-Ocampo[1]. 1) Dept Plant Pathology, North Carolina State University, Raleigh, NC; 2) Dept of Plant Biology, Michigan State University, East Lansing, MI.

Fusarium graminearum is a destructive pathogen of cereal crops such as corn where it causes stalk rot disease resulting in significant losses. Stalk rot reduces yield due to lodging of stalks, which limits production and harvesting of ears. Stalk rot may result in further losses since tissue can be contaminated with mycotoxins, making infected tissues unfit for food or feed. The main objective of this study was to identify candidate genes in both pathogen and plant with a potential role in disease, defense and mycotoxin production *in planta* using transcriptomics. For this, we inoculated a resistant and a susceptible corn varieties with *F. graminearum* wild type strain PH1 and carried out a time course experiment during the *F. graminearum*-corn interaction in stalks. We performed transcriptome analyses from RNA-seq data of the infected tissues and identified a set of genes differentially expressed in the pathogen and the host plant during stalk rot in reference to media grown *F. graminareum* and uninfected stalk tissues. Our findings highglight candidate genes that will benefit future molecular studies aimed at understanding the mechanisms of resistance and mycotoxin production in corn.

492. Interaction studies between *Fusarium graminearum* and *F. avenaceum* during durum wheat infection. Linda Harris, Danielle Schneiderman, Whynn Bosnich, Anne Johnston, Rachel Kwan, Barbara Blackwell, Adilah Bahadoor. Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, ON, Canada.

Numerous *Fusarium* species are pathogenic on Canadian cereals prior to harvest. These fungi are known to produce a diverse array of mycotoxins and other secondary metabolites which may act in synergy to affect plant resistance and metabolism and cause adverse effects on human and animal health. Recent surveys by the Canadian Grain Commission suggest that durum wheat samples are often contaminated with *Fusarium graminearum* and *F. avenaceum*, resulting in the deposition of trichothecenes, enniatins, moniliformin, and other bioactive compounds. To study the impact of *Fusarium* interactions, we chose two *F. avenaceum* strains (with sequenced genomes) and two *F. graminearum* strains (one 15-ADON- and one 3-ADON-producer) which were recently isolated from Canadian wheat samples. These

included one *F. avenaceum* and one *F. graminearum* strain which had been isolated from the same wheat sample. These strains were inoculated individually and in combination in durum wheat heads. Fungal biomass was monitored using species-specific digital PCR assays. Although *F. graminearum* greatly out-competed *F. avenaceum* in fungal biomass, we observed that co-inoculations of *F. graminearum* & *F. avenaceum* led to reduced disease and DON levels compared to single inoculations of *F. graminearum*. We also examined the transcriptome of *F. graminearum* grown *in vitro* in the presence of secondary metabolites extracted from *F. avenaceum* cultures. *F. graminearum* genes induced under these conditions included an ABC transporter gene, which was also induced in wheat heads only after *Fusarium* co-inoculations and not after single species inoculations. We are investigating whether this transporter is providing *F. graminearum* with a competitive advantage during interspecies interactions.

493. Degradation of the phytoalexin benzoxazolinones is important for virulence in *Fusarium* pathogens infecting wheat. Andrew Kettle[1,2], Jacqueline Batley[2,3], Aurelie Benfield[1], Jason Carere[1], John Manners[4], Kemal Kazan[1], Donald Gardiner[1]. 1) Agriculture flagship, C.S.I.R.O., Brisbane, Australia; 2) University of Queensland, School of Agriculture and Food Sciences, St. Lucia, Queensland, Australia; 3) University of Western Australia, School of Plant Biology, Crawley, Western Australia, Australia; 4) CSIRO Agriculture Flagship, Black Mountain, Canberra, Australian Capital Territory, Australia.

Fusarium-incited diseases of cereal crops, which include Fusarium head blight, Fusarium crown rot and Fusarium root rot, adversely affect food and feed production and threatens food security. A significant causal agent of these diseases is *Fusarium pseudograminearum*.

Benzoxazolinones, naturally occurring hydroxamic compounds, have been shown to have allelopathic, fungicidal, insecticidal and mutagenic activities. Outside the key role of these hydroxamic acids in cereal defence, fungal resistance to wheat defence chemicals are largely unknown.

In our research, we identified and functionally characterized members of the Fusarium detoxification of Benzoxazolinone (FDB) gene cluster that encode the enzymes for the pathway in the wheat pathogen *F. pseudograminearum*. Analyses of gene knockouts show that this gene cluster is essential for the detoxification of benzoxazolinones and phenoxazinones and contributes to pathogen virulence. To the best of our knowledge, this is the first report showing that benzoxazolinone detoxification ability limited to phenoxazinone production significantly contributes to the virulence of *F. pseudograminearum* specifically towards cereal hosts known to produce these defence compounds.

494. Computational identification of functional network modules associated with fumonisin biosynthesis in maize pathogen *Fusarium verticillioides*. Huan Zhang[1], Mansuck Kim[2], Charles Woloshuk[3], Byung-Jun Yoon[2,4], Won-Bo Shim[1]. 1) Plant Pathology & Microbiology, Texas A&M University, College Station, TX, 77843; 2) Electrical and Computer Engineering, Texas A&M University, College Station, TX,77843; 3) Botany & Plant Pathology, Purdue University, West Lafayette, IN, 47907; 4) College of Science, Engineering and Technology, Hamad Bin Khalifa University, Doha, Qatar, P.O.Box 5825.

Fusarium verticillioides is one of the key ear rot pathogens on maize. While a select number of genes associated with *F. verticillioides* virulence and mycotoxin biosynthesis have been characterized, our knowledge of the cellular and genetic network underlying these events is still very limited. In particular, we are struggling to understand the molecular signaling mechanisms that regulate fumonisin production during ear rot pathogenesis. In this work, we are performing a systematic network-based analysis of large-scale *F. verticillioides* RNA-seq data to identify potential gene modules that are responsible for ear rot pathogenesis and fumonisin regulation in the fungus. More specifically, our main goal is to identify subnetwork modules in the *F. verticillioides* co-expression network that show strong differential expression in two different maize lines (one moderately resistant and the other susceptible to the fungus), thereby identifying novel *F. verticillioides* pathways that are involved in virulence and fumonisin production. We first constructed the co-expression network of *F. verticillioides* using partial correlation, and searched through these networks to detect subnetwork modules that are differentially expressed in two different maize lines. We adopted the probabilistic pathway activity inference scheme to predict the activity level of potential subnetworks, and we developed a computationally efficient branch-out technique to find the subnetworks that display the largest differential expression. Through our analysis, we anticipate identifying potential modules, which consist of genes that show coordinated behavior during fumonisin biosynthesis and ear rot pathogenesis.

495. *Fusarium graminearum* is able to synthesize auxin and to inactivate the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). Gerhard Adam[1], Pia Spörhase[1], Anika Bartholomäus[1], Thomas Svoboda[1], Gerlinde Wiesenberger[1], Ulrich Güldener[2], Clemens Schmeitzl[1], Herbert Michlmayr[1], Philipp Fruhmann[1,3], Alexandra Parich[4], Bernhard Kluger[4], Hanswerner Mewes[2], Rudolf Krska[4], Rainer Schuhmacher[4], Eduardo Beltran Iturat[4], Franz Berthiller[4]. 1) Applied Genetics and Cell Biology, Univ. of Natural Resources & Life Sciences, Vienna, Tulln, Austria; 2) Department of Genome oriented Bioinformatics, Technische Universität München, 85354 Freising, Germany; 3) Institute of Applied Synthetic Chemistry, Vienna University of Technology, A-1040 Vienna, Austria; 4) Center for Analytical Chemistry, Department for Agrobiotechnology, BOKU. A-3430 Tulln, Austria.

In response to *Fusarium graminearum* infection the biosynthesis of tryptophan is upregulated in various plant hosts, presumably for production of tryptamine-derived defense compounds (e.g. coumaroyl/feruloyl-tryptamine). We found that *Fusarium* can intercept this process by efficiently converting tryptamine into the plant hormone auxin (indole-3-acetic acid), a negative regulator of plant defense. To elucidate which fungal genes are involved in this process we have expressed candidate Cu-amine oxidases from *F. graminearum* in baker's yeast, which is devoid of endogenous amine oxidase but is able to produce the prosthetic group needed for activity. Several gene products showed activity with tryptamine. Despite the redundancy in *F. graminearum*, a mutant (*aox4*) with only one inactivated gene showed a strong effect. Auxin production *in vitr*o was delayed and strongly reduced, while the wild-type converted tryptamine added to the medium rapidly to auxin with higher than 90% yield. High auxin levels normally trigger ethylene production in plants via the ACC pathway.

Interestingly, *F. graminearum* also contains two candidate genes annotated as putative ACC-deaminases, enzymes typically used by endophytes to downregulate ethylene-mediated defense responses. One of the *E. coli* expressed purified gene products showed indeed ACC deaminase activity. We conclude that *F. graminearum* has the potential to manipulate plant hormone levels relevant for plant defense.

496. A rapid assay for synthetic siRNA activity against TRI5 and DON production in *Fusarium graminearum* . Thomas Baldwin, Phil Bregitzer. Small Grains and Potato Research Unit, USDA, ARS, PWA, Aberdeen, ID.

Host Induced Gene Silencing (HIGS) has been demonstrated in multiple plant species as an effective and novel type of resistance to pathogens. This system functions via RNA interference (RNAi), which is initiated by double stranded RNA (dsRNA). RNAi can be initiated by Dicer-mediated production of siRNA 21mers derived from the dsRNA, or siRNA can be synthesized. Certain species of siRNA that are homologous to target pathogen gene sequences can suppress their expression. Hundreds of siRNA species can be derived from a long dsRNA, or single siRNAs can be synthesized. Here we directly test synthesized 21mer siRNAs for their effects against *TRI6* and *TRI10*, both transcriptional activators of *TRI5*, as revealed by GFP expression driven by the *TRI5* promoter in *Fusarium graminearum* strain *TRI5prom::GFP*. This strain also constitutively expresses RFP, which can be used as a measure of growth. The best induction of *TRI5* occurred with TBI media containing putrescine with a GFP fluorescence peak at 44 h post-inoculation. *TRI6*- siRNA 10 appeared to reduce GFP detection up to 50%. Other siRNAs directed against *TRI6* were no effective at reducing GFP expression. Two siRNAs (4 and 13) directed against *TRI10* appeared to reduce GFP expression. Surprisingly, siRNAs 9 and 11 against *TRI10* resulted in increased GFP expression. These results, based on preliminary experiments, require further verification. siRNAs showing consistent, positive effects will be used as a basis to design constructs for transformation of *F. graminearum* and for barley.

497. Saprophytic growth of *Fusarium graminearum* on inactive wheat heads produces different transcriptional and metabolite profiles compared to pathogenic growth. S. Boedi[1], C. Sieber[3], M. Münsterkötter[3], H. Berger[1], T. Nussbaumer[3], K. Kugler[3], I. Maloku[2], M. Sulyok[2], V. Preiser[2], G. Siegwart[2], E. Sam[2], M. Lemmens[2], K. Brunner[2], U. Güldener[3], J. Strauss[1]. 1) Fungal Genetics and Genomics Unit, Department of Applied Genetics and Cell Biology, BOKU University and Austrian Institute of Technology GmbH, University and Research Center Tulln (UFT), Konrad Lorenz Strasse 24, A-3430 Tulln; 2) Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria; 3) Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg.

Traditionally, control samples for infection experiments are derived from submerged fungal cultures grown on either minimal or complex liquid medium. As these media are highly different from the substrate the fungus encounters during wheat infection, pathogen-related differentially regulated genes may simply respond to nutritional differences. To clarify these aspects we compared transcriptome and metabolic profiles from cultures grown on active and inactive wheat heads and in synthetic minimal media. Within a wheat infection experiment applying *F. graminearum* Ph-1 spores to the susceptible cultivar *Remus* one set of ears was inoculated on the living plant representing pathogenic growth of the fungus. To gain “as similar as possible” control samples, another set of wheat heads, which were cut off the plant prior to spore application, was inoculated as “non- response” control representing saprophytic growth of the fungus on an identical substrate. Additionally, Ph-1 was cultured in minimal medium containing the DON inducing nitrogen source L-ornithine. Chemical analysis revealed induction of mycotoxin production during infection of living wheat heads and in the L-ornithine medium, whereas mycotoxin levels remained low after saprophytic growth. RNA- Seq analysis of these samples revealed that putative pathogenicity-related genes differentially regulated between saprophytic and pathogenic growth are mainly responding to nutritional conditions. However, some of these de-regulatred genes appear to be independent from the substrate and may allow new insights into pathogenicity of the fungus.

498. Comparison of the resistance mechanisms to *Fusarium gaminearun* infection in two resistant wheat cultivars. Ludmila Roze, Josh Springer, Frances Trail. Plant Biology, Michigan State University, East Lansing, MI.

Infection of wheat heads by *Fusarium graminearum* initiates the head blight disease, which results in mycotoxin contamination of harvested grain. The spread and colonization of the head during infection was examined in several wheat cultivars by microscopy using a GFP-tagged strain. The host response to the fungal infection in resistant cultivars was shown to be associated with limitation of fungal growth and blockage of fungal penetration into the cells. Histochemical investigation indicated that the response was accompanied by massive depositions of phenolic compounds associated with the fungal hyphae in the upper portions of the rachis fragment adjacent to the inoculation point. Also, accumulation of pectin within and along the vascular bundles, within parenchymatous cells was observed. The resistance response differed between the cultivars, with one cultivar demonstrating a stronger resistance response in comparison to the other. Strong resistance to *F. graminearum* has been difficult to introduce in wheat and this investigation helps to distinguish resistance responses in two cultivars.

499. Interplay of MAPK and ROS signaling in chemotrophic growth of *Fusarium oxysporum*. Daniela Dirschnabel, David Turrà, Antonio Di Pietro. Department of Genetics, University of Córdoba, Córdoba, Spain.

Chemotropism, the ability to re-orient the growth axis in response to chemical cues, is critical for many aspects of fungal lifestyle such as colony establishment, foraging for nutrients or location of host organisms. We use the root-infecting pathogen *Fusarium oxysporum* as a model to study various aspects of chemotropic signaling such as the perception of chemoattractants from the host plant. Previous studies revealed that chemotropism towards nutrients, sex pheromones or plant signals is governed by distinct mitogen-activated protein kinase (MAPK) cascades. The major chemoattractant secreted by tomato roots was identified as a peroxidase, an enzyme that catalyzes the reductive cleavage of H_2O_2. Together with the observation that chemoattraction towards tomato roots is abolished by the antioxidant ascorbate, this finding points towards a role of reactive oxygen species (ROS) in activation of the chemotropic response. The ROS-generating enzymes NADPH oxidases (Nox) were previously shown to regulate developmental processes requiring chemotropic growth such as hyphal fusion and plant infection. We have created deletion mutants in the NADPH oxidases NoxA or NoxB, as well as in the

associated regulator NoxR. The phenotypes of these mutants are currently being characterized in order to determine the impact of NOX in MAPK-mediated chemotropic growth of *F. oxysporum*..

500. *Fusarium oxysporum* f. sp. *apii* on celery in California, USA. Lynn Epstein, Sukhwinder Kaur, Peter Chang, Noelia Carrasquilla-Garcia, Douglas Cook, Krishnamurthy Subbarao. Plant Pathology, University of California, Davis, CA.

Fusarium oxysporum f. sp. *apii* (*Foa*) race 1 was first reported on celery with Fusarium yellows in 1914; it is virulent on self blanching celery cv. Golden but avirulent on green cultivars, e.g. Tall Utah. In 1976, *Foa* race 2 was identified in California; it is virulent on cv. Tall Utah, but asymptomatic to weakly virulent on cultivars (e.g. cv. Challenger) with the celeriac-derived gene *Fu1*. A race 3 from California that is virulent on Tall Utah but avirulent on both cv. Golden and cv. Challenger also was reported. Between 1993 and 2014, we isolated *F. oxysporum* from celery crown and root tissue that had symptoms of Fusarium yellows, primarily from California. DNA sequence (628 bp from EF-1α and 640 bp from rDNA IGS) were determined for 181 isolates. Between 1993 and 2012, all isolates that caused the iconic orangish-brown discoloration were a clonal population of *Foa* race 2 with a monomorphic sequence that has not been reported previously. In addition to *Foa* race 2, a diversity of *F. oxysporum* that do not cause symptoms were frequently isolated from the margin of symptomatic tissue; 82% (n=56) were in "clade 5" and 9% were in "clade 1." In 2013, a new race 4 was discovered in three celery production fields in Camarillo, California; race 4 isolates are highly virulent on both Tall Utah and the race 2-resistant Challenger. Based on the EF-1α and rDNA IGS amplicons, race 4 is related to race 3, which is in "clade 1," as are all the previously described *Foa*. Next generation sequencing was done with Ilumina Mi-Seq paired-end reads to 10X coverage on 15 of the isolates (six race 2 isolates selected over time and location), one isolate of races 4 and 1, and six and one of the polymorphic non-pathogenic clade 5 and clade 1 isolates, respectively. Sequences were assembled primarily against the *F. oxysporum* full-genome sequenced strains at the Broad. Analysis of SNPs with *structure*, Nei's and Fst indicate that race 4 and race 2 are relatively unrelated, that the polymorphic non-pathogenic isolates in clade 5 are in a distinct clade, and that race 2 has been relatively invariant in California between 1993 and 2014. Results on race-specific detection and quantification and further phylogenetic analyses will be presented.

501. Comparative analysis of *F. oxysporum* transcriptomes during infection of a resistant or susceptible host of the model legume species *Medicago truncatula*. Louise Thatcher[1], Angela Williams[1], James Hane[1,2], Gagan Garg[1], Judith Lichtenzveig[2], Karam Singh[1,3]. 1) Agriculture Flagship, CSIRO, Floreat, WA, Australia; 2) Centre for Crop and Disease Management, Curtin University, Bentley, WA, Australia; 3) The Institute of Agriculture, University of Western Australia, Crawley, WA, Australia.

Fusarium wilt disease caused by the root-infecting fungal pathogen *Fusarium oxysporum* is a major constraint on grain legume production, and in Australia represents a significant biosecurity threat to this industry. To gain insight into the molecular interaction between *F. oxysporum* and its legume hosts we have been using the model legume species *Medicago truncatula* and conducted high coverage RNA sequencing (RNAseq) of infected Medicago roots to detect potential *F. oxysporum* effector genes expressed during infection of a resistant or susceptible Medicago cultivar. In combination with RNAseq on an *in culture* sample, this process identified new putative effector genes as well as previously identified effectors such as some members of the *F. oxysporum* f.sp. *lycopersici Secreted In Xylem* (*SIX*) effectors. While some putative effectors were only expressed reliably under one condition, the majority were expressed in both resistant and susceptible hosts but with varying potency. Possible mechanisms of *F. oxysporum* f.sp. *medicaginis* pathogenicity and virulence will be discussed.

502. Functional characterization of *Fusarium oxysporum* effectors using Arabidopsis as host plant. Nico Tintor[1], Peter van Dam[1], Libera Lo Presti[2], Regine Kahmann[2], Martijn Rep[1]. 1) Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands; 2) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

Fungi are common plant colonizers, thereby instigating in some cases disease symptoms. Colonization success largely depends on the ability to manipulate the host plant, often achieved via effectors, secreted proteins that act either inside plant cells or in the apoplast. *Fusarium oxysporum* is a soil inhabiting fungus that can infect many plant species via their roots. Fusarium effectors were previously characterized as small, secreted proteins that accumulate in the xylem during infection. However, the mechanism by which these effectors contribute to virulence remains unknown. Also, it is unclear for most Fusarium effectors whether they act inside or outside plant cells.

Using the Arabidopsis – Fusarium pathosystem we are aiming to identify the functional sites and virulence mechanisms of effectors. We generated a shortlist of ca. 20 candidate effectors from an Arabidopsis infecting Fusarium strain, based on homology to known effectors and presence of a 'miniature impala' (mimp) transposable element in the promoter. To investigate which of these effectors are translocated to living cells during infection, we apply an *in planta* biotinylation assay. Candidate effectors were C-terminally fused to a short tag that serves as a biotin-acceptor site inside plant cells and transformed into Fusarium. We robustly detect several candidate effectors at the protein level during infection of Arabidopsis roots, and are currently examining if specific biotinylation of these effectors occurs. Effectors of interest will be further characterized for their virulence function and putative plant interactors. This method might facilitate the identification of novel effectors acting in plant cells as well as their virulence targets.

503. Is SIX1 an effector in the *Fusarium oxysporum* f.sp *cubense*-banana? Sri Widinugraheni[1], Jonathan Niño Sánchez[2], H.C van der Does[1], Corby Kistler[2], Martijn Rep[1]. 1) SILS, University of Amsterdam, Netherlands; 2) USDA-ARS Cereal Disease Laboratory, St. Paul, Minnesota.

Fusarium oxysporum f.sp. *cubense* (Foc) causes wilt disease in banana (known as Panama disease). This fungus exists in several different races. Of the banana we eat as a fruit, Race 1 infects only Gros Michel and not Cavendish (our current banana), but Race 4 can infect all commercial banana cultivars. Studies of *Fusarium oxysporum* f.sp. *lycopersici* (Fol), a pathogen towards tomato, has given insight in *Fusarium oxysporum*-host interactions. In this interaction, small secreted proteins (Six, Secreted in Xylem) are important. Six proteins can act as a virulence factors, but may also trigger resistance in the plant host. In tomato, three resistance genes are known, *I-1*, *I-2* and *I-3*, and

the corresponding *Avr* genes *SIX4 (Avr1), SIX3(Avr2), SIX1(Avr3)* have been identified (Takken and Rep, 2010). In Foc, however, little is known about the effectors necessary to infect banana, or perhaps triggering immune responses in some cultivars. There are three homologs of *SIX1* in Foc Tropical Race4 (TR4), designated SIX1a,b,c. We wanted to know whether the *SIX1* homologs from Foc can fulfill the avirulence function of Fol *SIX1*. To test that, Fol *SIX1* is replaced by Foc *SIX1* homologs and inoculated I-3 tomato resistant plants with the Fol transformants. Our data show that *SIX1c*, and possibly *SIX1a*, can be recognized by the *I-3* resistance gene in tomato. Further, we asked whether *SIX1* has a virulence function during banana infection. Result from an infection assay of Cavendish with Foc TR4 wild type and a Foc delta *six1a* mutant, suggests that *SIX1a* has a virulence function on Cavendish.

504. Involvement of ABC transporters in xenobiotic tolerance and antagonism in the fungal biocontrol agent *Clonostachys rosea*. Mukesh Dubey, Dan Funck Jensen, Magnus Karlsson. Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

ATP-binding cassette (ABC) transporters mediate active efflux of natural and synthetic toxicants, and are considered to be important for drug tolerance in microorganisms. In biological control agents (BCAs), ABC transporters can play important roles in biocontrol mechanisms by providing protection against metabolites derived from the fungal prey, host plant, and by mediating the secretion of endogenous toxins. The fungus *Clonostachys rosea* is a highly efficient BCA against fungal pathogens to crops. In addition, *C. rosea* can tolerate higher concentrations of chemical fungicides, in relation to doses recommended for controlling fungal diseases of plants. In this work, by generating gene deletion strains, we characterize the biological functions of two ABC transporters ABCG5 and ABCG29 that were previously identified as *Fusarium* mycotoxin zearalenone (ZEA)-induced genes in *C. rosea* strain IK726. Gene expression analysis shows induced expression of *abcG5* and *abcG29* in presence of ZEA, *F. graminearum* culture filtrates and different classes of fungicides. Phenotypic analysis showed that *abcG5* deletion strains were more sensitive towards ZEA, iprodione- and mefenoxam-based fungicides and had reduced antagonism towards *F. graminearum* in an *in vitro* dual culture assay. In contrast with gene expression data, *abcG29* deletion strains did not show differences in tolerance to ZEA or fungicides, or in antagonism compared to *C. rosea* WT. This contradiction may be related with overlap in substrate specificity of ABCG29 with other *C. rosea* ABC transporters, as the *C. rosea* genome contains 86 ABC transporters that may complement the function of ABCG29. Furthermore, *in vivo* sand seedling tests showed that both Δ*abcG5* and Δ*abcG29* strains had reduced ability to protect barley seedlings from *F. graminearum* foot rot disease. Taken together, these results show that ABC transporters are important for xenobiotic tolerance and biocontrol traits in *C. rosea*.

505. Molecular manipulation of the mating-type system for the development of fungal populations in *Pyrenophora tritici-repentis*. Z. Liu[1], G. Ameen[1], T. Friesen[1,2], J. Faris[2]. 1) Department of Plant Pathology, North Dakota State University, Fargo, ND 58108; 2) USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND 58105.

The ascomycete *Pyrenophora tritici-repentis* (*Ptr*) is a devastating fungal pathogen worldwide that causes tan spot of wheat. The fungus is self-fertile (homothallic) because each individual contains both mating type (*MAT*) idiomorphs. In this work, we developed knockouts of *MAT* genes in *Ptr* and tested fertility of the knockout strains and the crosses between the knockout strains carrying the opposite mating type. The results showed that the deletion of either *MAT1-1* or *MAT1-2* leads to complete sterility of the fungus without the formation of mature pseudothecia, but the crosses between two mating type gene knockouts result in the production of normal pseudothecia and ascospores. Using a cross between 86-124 (*ToxA*-containing isolate) and DW5 (*ToxB*-containing isolate), we developed a fungal population that segregated for the *ToxA* and *ToxB* genes but with a non 1:1 ratio. Greenhouse inoculations using fungal progenies demonstrated that virulence of each progeny on the tan spot differentials was correlated with the presence of the two genes. This work provides a novel and effective means to further characterize fungal pathogenicity/virulence in *Ptr* through the development of segregating fungal populations and the utilization of genetic analyses.

506. Identification of major lipases in cutaneous pathogenic yeast *Malassezia restricta*. Minji Park[1], Yang Won Lee[2], Yong-Joon Cho[3], Won Hee Jung[1]. 1) System Biotechnology, Chung-Ang University, Anseong-si, Gyeonggi-do, South Korea; 2) Department of Dermatology, School of Medicine, Konkuk University, Seoul, 143-729, Korea; 3) School of Biological Sciences & Institute of Bioinformatics, Seoul National University, Seoul, Republic of Korea.

Malassezia species are basidiomycetous yeast and opportunistic pathogens often associated with seborrheic dermatitis including dandruff. To date, 14 *Malassezia* species have been identified, and among them, *M. restricta* is the most frequently isolated species from the healthy and disease skin. Almost all of the *Malassezia* species are lipophilic, of which property might be compensated by breaking down the sebum into fatty acid using lipases or phospholipases. Indeed, the recent genome analysis revealed that *M. globosa* possesses 14 lipases although a role of each enzyme is poorly described. In this study, we aimed to evaluate expression of the genes that encode a lipase homolog in *M. restricta* on scalp of the patients with dandruff and to identify the gene that is expressed upon interaction with the human host. Sequences of total 11 lipase homologs of *M. restricta* were obtained by sequencing of the *M. restricta* genome, and their transcript levels were evaluated in swap samples from 57 patients with severe dandruff. We found that genes encoding the lipase homolog, MRES_21260 and MRES_35760, were expressed most predominantly in the samples while only few samples displayed expression of other homologs. These results suggested that MRES_21260 and MRES_35760 are the major lipase homologs in *M. restricta* that is expressed on the skin. These two lipases and MRES_33300, which is a homolog of *LIP1* known as major lipase in *M. globosa*, were subsequently overexpressed in *Pichia pastoris*. Purified proteins were used for characterization of the enzymes and antibody generation. The results of our study will contribute to understand the interaction between *Malassezia* and the host.

507. Orchid mycorrhizae: The Next Generation (Sequencing). Sarah Unruh[1], Lawrence Zettler[2], Logan Decker[1], Patrick Shiu[1], J. Chris Pires[1]. 1) Biological Sciences, University of Missouri-Columbia, Columbia, MO; 2) Biology, Illinois College, Jacksonville, IL.

Orchids associate with a large diversity of fungal species. Their mycorrhizal relationship is unique because the fungi supply the plants with carbon while there is no documented plant to fungus transfer of nutrients. Understanding this relationship is important because many

of these fungi have other roles in the environment—decomposers, ectomycorrhizal associations with other plants—and other species in certain orchid fungi genera are plant pathogens. Knowing more about the molecular nature of this partnership may shed light on many more processes involving plant/fungal interactions. To study this orchid/fungal partnership we will use next-generation sequencing technologies for gene discovery, expression-level analysis, and gene family evolution in the fungi, the orchids, and both in symbiotic culture. We are working with Lawrence Zettler who has isolated more than 400 fungi from wild orchids. These samples have been identified with ITS to belong to three main genera that form orchid mycorrhizas (*Tulasnella, Ceratobasidium,* and *Sebacina*). In collaboration with the Shiu lab we are growing these fungal cultures to extract genetic material. The first step is to sequence the genomes of representative fungal species. In addition, we will produce transcriptome data of the same fungal cultures and map those reads onto our genomic sequences to build a more robust genome assembly. The RNAseq data will also be used as a comparison for expression studies of symbiotic orchid seedlings. These "–omics" resources will contribute to our understanding of mycorrhizal associations with potential implications for pathogenesis research.

508. *In vivo* visualisation of fungal infections: An introduction to strain construction, imaging and challenges. Matthias Brock. Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knoell Inst, Jena, Germany.

In recent years several advances have been made to visualise the infection process from fungal pathogens under *in vivo* conditions. *In vivo* imaging allows the investigation of time-resolved interactions of a pathogen with its host and provides temporal and spatial information on disease progression or successful clearance. Fluorescence and bioluminescence imaging make up the major proportions of currently used *in vivo* imaging techniques, but all systems have advantages and disadvantages that need to be analysed prior to selection of a specific system. While fluorescence imaging is the method of choice in intravital microscopy or the investigation of *in vivo* host pathogen interactions in transparent animals such as the zebrafish model, high background fluorescence significantly impacts its use in murine infection models. Therefore, investigation of disease progression and therapy monitoring in murine models mainly relies on the use of light-emitting luciferases. Although the spatial resolution from bioluminescence imaging is far below the resolution from fluorescence imaging, it is an extremely sensitive method due to very low background signals. To introduce the power of bioluminescence imaging, monitoring of invasive aspergillosis and disseminated candidiasis in temporal and spatial resolution will be shown. Additionally, suggestions will be given that may be suitable to increase the sensitivity of bioluminescence imaging systems and its potential use in studying disease caused by plant pathogenic fungi.

509. MoPos5, a NAD(H) kinase regulates detoxification of reactive oxygen species and virulence in *Magnaporthe oryzae*. Xiaofeng Chen[1], Wenhui Zheng[1], Renli Cai[1], Zonghua Wang[1], Daniel Ebbole[2], Guodong Lu[1]. 1) The Key Laboratory for Bio-pesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002, China; 2) Departmant of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA.

NAD(H) kinase plays a major role in protecting living cells against oxidative stress through regulating the fine intracellular balance between NAD(P) and NADP(H). Host-driven production of reactive oxygen species (ROS) acts as a major barrier for the colonization of host tissue by plant pathogens. However, it is unclear how NAD(H) kinase participates in the defense against ROS in plant pathogens. In this study, we identified and characterized MoPos5, a homolog of the *Saccharomyces cerevisiae* NAD(H) kinase POS5 in *Magnaporthe oryzae.* The MoPos5 protein contains typical conserved motifs and domains of NAD(H) kinase, and can functionally complement the growth deficiency of the *POS5* deletion mutant in *S. cerevisiae* under oxidative stress. *MoPos5* was expressed in all life stages of the fungus, and during its colonization and proliferation *in planta.* The MoPos5-eGFP fusion protein was localized inside the mitochondria. The deletion of the *MoPos5* gene in *M. oryzae* resulted in severe vegetative and conidiation defects, however, the conidial morphology, germination and appressoria development were normal in comparison to the wild-type strain. MoPos5 is essential for utilizing linolenic acid as carbon source, which is likely due to its role in NADPH generation that is necessary for β-oxidation of linolenic acid. In addition, the null mutants successfully penetrated rice leaf sheath cells, however, they failed to colonize host tissues.

510. The interactome of pathogenicity factors in the rice blast fungus *Magnaporthe oryzae*. Yang Li[1], Xiaoying Zhou[1], Keerthi B Jayasundera[2], Anton Iliuk[2], Huiquan Liu[3], Andy Tao[2], Jin-Rong Xu[1,3]. 1) Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN; 2) Dept. of Biochemistry, Purdue University, West Lafayette, IN; 3) Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, China.

Rice blast is a disease of significant economic impact worldwide and a model system for studying fungal-plant interactions. In *Magnaporthe oryzae*, over 100 genes have been identified to play important role in infection-related growth and pathogenecity. Some of them shared common signaling pathway and biochemical processing. However, a systematic functional characterization of the pathogenecity factors and their interactions has never been reported. Mapping protein-protein interaction networks of the identified pathogenecity factors in *M. oryzae* facilitate to reveal molecular mechanisms regulating plant infection processes and to discover novel pathagenecity factors. In this study, we characterize the interactome of over 70 identified pathogenecity factors by taking advantage of the affinity purification combined with proteomics approaches. Protein-protein interaction maps were established for these pathogenicity factors. Co-immuniprecipitation, and BiFC assays were used to verify the interactions of selected genes. Based on our protein-protein interaction maps, we also have identified twelve novel functional genes related to pathogenicity in *Magnaporthe oryzae*. So far, it is the first study to functionally characterize the pathogenecity factors by mapping them in protein-protein interaction networks in plant pathogenic fungi *M. oryzae*.

511. GATA-dependent glutamine metabolism links cAMP/ cPKA- and TOR-signaling pathways to drive appressorium formation by *Magnaporthe oryzae*. Margarita Marroquin-Guzman, Richard Wilson. Plant Pathology Department, University of Nebraska-Lincoln, Lincoln, NE.

Rice blast infection begins when an asexual spore of *Magnaporthe oryzae* lands on the surface of a rice leaf, germinates and develops a

specialized infection structure called an appressorium. The cAMP/ PKA- and MAP kinase-signalling cascades have been functionally described as positive-acting pathways required for appressorium development. Negative-acting regulatory pathways that block appressorial development are not known. Previously, the GATA-family transcription factor, Asd4, was shown to be required for appressoria formation and pathogenicity. Using genetic and biochemical approaches, we attempted to identify the underlying mechanisms governing Asd4-dependent appressoria formation, and how they might connect to other signaling pathways. *Δasd4* mutant strains could not form appressoria and expressed *GLN1*, a glutamine synthetase-encoding orthologue, which is normally silent in wild type and results in elevated intracellular glutamine levels in *Δasd4* mutants. Deleting *GLN1* in *Δasd4* restored intracellular glutamine levels and allowed appressoria formation. Moreover, appressorial development was restored in *Δasd4* mutants by adding rapamycin, the specific inhibitor of the Target of Rapamycin (TOR) signaling pathway, a pathway activated in yeast in response to glutamine sufficiency signals. However, appressorium formation in *Δasd4* mutant strains was not restored by treatment with monobutyryl cyclic AMP (cAMP), placing TOR downstream of the cAMP/cPKA signaling pathway during appressorium development. Together, these results suggest that the manipulation of cellular glutamine metabolism by Asd4 controls appressorium formation via the TOR signaling pathway. This is the first report indicating that TOR is a negative-acting regulator of appressoria formation in *M. oryzae*, thus adding to our knowledge about how these specialized infection cells develop during fungal-plant interactions.

512. *Magnaporthe oryzae* effectors with putative roles in cell-to-cell movement during biotrophic invasion of rice. Pierre A Migeon[1], Mihwa Yi[2], Xu Wang[3], Jung-Youn Lee[3], Barbara Valent[1]. 1) Interdepartmental Genetics Program, Kansas State University, Manhattan, KS; 2) Samuel Roberts Noble Foundation, Ardmore, OK; 3) Department of Plant and Soil Sciences, University of Delaware, Newark, DE.

The rice pathogen *Magnaporthe oryzae* grows intracellularly within living rice cells and invades subsequent cells by undergoing extreme constriction as it passes through the host cell wall. We have identified six biotrophy-associated secreted (BAS) proteins that accumulate around invasive hyphae at the points where they cross the plant cell wall and have designated these effectors as putative fungal movement proteins (fMPs). In order to better understand their putative role in assisting cell-to-cell crossing of the fungus during biotrophic infection, live cell fluorescence microscopy has been performed to characterize localization and dynamics of these effectors during biotrophy. Previous studies have demonstrated distinct golgi-dependent and golgi-independent secretion pathways of *M. oryzae* BAS effectors correlating with localization to either the host-pathogen interface or host cytoplasm (respectively). *M. oryzae* is separated from the rice cytoplasm by host derived membrane, and must maintain this membrane as it passes into subsequently invaded cells. Using the golgi inhibitor Brefeldin A and FRAP experiments, we have obtained evidence for unique apoplastic dynamics of the putative fMPs. We have taken an RNA silencing approach to characterize the roles of these effectors and have developed a biotrophy-specific hairpin silencing construct using an endogenous highly expressed BAS promoter towards this end.

513. Appressorium formation of *Pyricularia oryzae* as a sensitive drug target analysis system. Akira Ishii, Mayu Kumasaka, Syunta Kusama, Akihito Nozaka, Kentaro Ikeda, Jesus Izaguirre-Carbonell, Megumi Narukawa-Nara, Fumio Sugawara, Takashi Kamakura. Applied Biological Sciences, Tokyo University of Science, Noda, Chiba, Japan.

We aimed to establish a novel approach to identify targets for drugs using fungi, *Pyricularia oryzae* that is the causal agent of rice-blast disease. *P. oryzae* differentiates specialized infection structure known as an appressorium. Since the developmental stage of appressorium is sensitive to various pesticides, we utilized the cellular differentiation of *P. oryzae* for an indicator of inhibitory effects caused by various chemicals. Using the *P. oryzae*, we searched a novel molecular target of Roxithromycin (RXM), a macrolide antibiotic, which was primarily target the 50S ribosomal subunit of prokaryote. RXM is known to show some beneficial side effects to eukaryotes, though the mechanisms of these effects remain uncertain. We found RXM showed specific inhibition to appressorium formation of *P. oryzae*, indicating that there is an alternative target of RXM in *P. oryzae*. We chose T7 phage display as the highly sensitive method to search the interaction between immobilized compound and peptide displayed on T7 phage's virion. We identified the PoCDC27 (*P. oryzae* cell division cycle 27 homolog) as a candidate target of RXM. PoCDC27 probably is related to the cell differentiation. We generated knockdown and overexpression mutants of PoCDC27 to study how RXM affects appressorium formation of *P. oryzae*. The *pocdc27* knockdown mutants were less sensitive against RXM of wild type, and overexpression mutants were sensitive to RXM as much as wild type. The complement mutants restored the wild type phenotype. These results suggested that PoCDC27 interacted with RXM *in vivo*. Moreover, the interaction of RXM-PoCDC27 was also studied by using surface plasmon resonance sensors *in vitro*. From both *in vivo* and *in vitro* experiments, we found that the complex of RXM-Pocdc27 affected another molecule involved in appressorium formation. In this study, we found the novel candidate target of RXM in *P. oryzae*. We infer this approach has a remarkable potential for understanding the mechanisms of drug. It will lead to develop new drugs and to appreciate the novel application of *P. oryzae* as an experimental tool.

514. Expression of *HopAI1* interferes with MAP kinase signaling pathways in *Magnaporthe oryzae* . X. Zhang, WD. Liu, JR. Xu. Botany and plant pathology, Purdue University, West Lafayette, IN.

Pseudomonas syringae delivers effector proteins into plant cells by the bacterial type III secretion system (TTSS). Some of these *P. syringae* effectors target fundamental cellular processes that are conserved among eukaryotes. In this study, we expressed 2 T3SS effectors *HopAI1* and *HopI1* effectors in the rice blast fungus *Magnaporthe oryzae*. Whereas expression of *HopI1* had no obvious effects, expression of *HopAI1* with different promoters affected various development and infection processes. *HopAI1* is a phosphothreonine lyase that possesses the same *in vitro* catalytic activity as OspF and SpvC on Erk2 MAP kinases. In *M. oryzae*, over-expression of *HopAI1* under the control of constitutive promoters *RP27* resulted in defects in conidiation and hyphae growth similar to that of the Δ*mps1* mutant. The phosphorylation level of both Pmk1 and Mps1 was reduced in the *HopAI1* transformants. Co- immunoprecipitated with *Mps1* and *Pmk1* shows physical interaction between *HopAI1* and *Mps1*. To determine the effects of stage-specific expression of *HopAI1*, we used the *BAS1* and *MIR1* promoters to express *HopAI1* in *M. oryzae*. While *MIR1* is highly up-regulated during plant infection, and *BAS1* are specifically expressed in appressoria and invasive hyphae, respectively. P_{BAS1}- or P_{MIR1}-*HopAI1* transformants were normal in growth, conidiation, and appressorium formation but reduced in appressoria penetration and virulence. In rice leaf sheath penetration assays, the P_{BAS1}- or P_{MIR1}-

HopAI1 transformants were defective in invasive growth, indicating MPAK signaling is important for cell-to-cell movement of infectious hyphae.

515. The Vps35/retromer is essential for appressorium-mediated host infection by the rice-blast fungus *Magnaporthe oryzae*. Wenhui Zheng[1], Jie Zhou[1], Yunlong He[2], Qiurong Xie[1], Ahai Chen[1], Huawei Zheng[1], Lei Shi[1], Xu Zhao[1], Chengkang Zhang[1], Qingping Huang[1], Kunhai Fang[1], Guodong Lu[1], Daniel Ebbole[3], Naweed Naqvi[2], Zonghua Wang[1]. 1) Key Laboratory of Bio-pesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China; 2) Temasek Life Sciences Laboratory and Department of Biological Sciences, National University of Singapore, Singapore; 3) Department of Plant Pathology and Microbiology, Texas A&M University, College Station, USA.

The retromer mediates cargo selection in the endosomal protein sorting, retrieval and transport machineries in eukaryotes. However, the role of such membrane trafficking events during pathogen-host interaction remains unclear. The rice blast fungus, *Magnaporthe oryzae,* invades the host plants using key infection structures, called appressoria that are elaborated at the tips of the conidial germ tubes. Here, we report that the retromer subcomplex (MoVps35, MoVps26 and MoVps29) is essential for appressorium-mediated host penetration in *M. oryzae*. Loss of retromer function led to blocked glycogen distribution, turnover of lipid droplets, delayed nuclear degeneration in conidia, and reduced turgor development in the appressoria. The aforementioned defects in *ΔMovps35* were reminiscent of the *atg8*-deletion in *M. oryzae.* Interestingly, the *ΔMovps35* mutant showed significantly reduced lipidation of MoAtg8 upon nitrogen starvation; and was consequently impaired in the biogenesis of autophagosomes (RFP-Atg8) in the mutant germ tubes and appressoria. Live-cell imaging revealed highly mobile MoVps35-GFP puncta in close association with RFP-Atg8 in the perivacuolar region. MoVps35-GFP physically interacted with cleaved RFP-Atg8 *in vivo*. Taken together, our data support a key and essential role for MoVps35/retromer in regulating the autophagy process, likely via retrieval and vacuolar membrane sorting, during the initiation of blast disease in rice.

516. Protease-induced apoplastic defense signaling in *Zea mays*. Sebastian Ziemann[1], Karina van der Linde[2], Renier van der Hoorn[3], Farnusch Kaschani[4], Nick Holton[5], Cyril Zipfel[5], Gunther Doehlemann[1,2]. 1) Botanical Institute and Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, BioCenter, Zuelpicher Str. 47a, 50674 Cologne, Germany,University of Cologne, Cologne, Germany; 2) Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strabe 10 D-35043 Marburg, Germany; 3) Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK; 4) University of Duisburg-Essen, D-45117 Essen, Germany; 5) The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK.

Papain-like cysteine proteases play important roles in plant defense mechanisms. During the interaction of the biotrophic corn smut fungus *Ustilago maydis* with its host *Zea mays,* protease profiling revealed a significant inhibition of maize cysteine protease activity. *U. maydis* suppresses the activation of salicylic acid (SA)-dependent defense genes by inducing the plant compatibility factor CC9 (corn cystatin 9). CC9 blocks SA-signaling by inhibition of apoplastic cysteine proteases (ACPs). In turn, activated ACPs induce SA-associated defense gene expression in naïve plants, demonstrating a crucial role of maize cysteine proteases in SA-dependent defense signaling.

Based on these findings, we aimed to identify signals that are released by ACPs to induce plant defense. In a mass spectrometry analysis we discovered a maize peptide (now termed DAMP2) being highly enriched in SA treated apoplastic fluid. DAMP2 induces *PR*-gene expression as well as ACP activity, when applied to maize leaves. Recombinantly expressed precursor-protein (ProDAMP2) is cleaved by maize ACPs *in vitro* and releases *PR*-gene inducing peptides. This demonstrates that ACP activity releases DAMP2, a specific signal peptide that triggers SA-associated defense responses in maize.

Here we present recent progress on DAMP2 mediated signaling as well as the results of an approach to identify host receptors for DAMP2.

517. Transcriptional program of the yeast-mycelium dimorphism in a non-model vascular plant pathogen. Martha Nigg[1,2], Christian R Landry[2,3], Louis Bernier[1,2]. 1) Center of Forest Research (CFR), Université Laval, Quebec, Canada; 2) Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Quebec, Canada; 3) Biology Department, Université Laval, Quebec, Canada.

The highly aggressive fungus *Ophiostoma novo-ulmi* is one of the causal agents of Dutch elm disease, a vascular wilt in which the fungus colonizes the xylem vessels and ultimately kills its host. This fungus is dimorphic, i.e. it has the capacity to grow either in yeast or in mycelial form. During the infection process, the yeast phase is used for passive colonization of elm xylem vessels, whereas the mycelium form (also called the invasive phase) allows for lateral spread into adjacent vessels. Thus, dimorphism is thought to be a key morphological characteristic for fungal fitness and disease development. The recently sequenced genome of *O. novo-ulmi* ssp. *novo-ulmi* strain H327 offers a rich source of information that can be used to investigate the regulatory program of this morphological and life-history switch. In some dimorphic model organisms such as *Saccharomyces cerevisiae, Candida albicans* and *Ustilago maydis,* at least three different and highly conserved pathways are known to be involved in the regulation of yeast to mycelial switch in response to environmental stimuli: i) a Mitogen-Activated Protein Kinase (MAPK) cascade; ii) the cyclic AMP-dependent protein kinase (cAMP-PKA) pathway; and iii) the pH-induced Pal/RIM pathway. Comparative genome analyses show evidences that homologs of these regulatory pathways are present in the *O. novo-ulmi* genome. Using high throughput RNA sequencing, we are examining whether these three conserved and also other pathways may be involved in the *O. novo-ulmi* morphological switch. A preliminary experiment on distinct yeast and mycelial forms allowed to characterize the global gene expression profiles of the two phases. Results from ongoing analyses will 1) highlight the molecular pathways that are differentially expressed between the two phases; 2) test for the role of the MAPK, PKA and RIM pathways in the yeast-mycelium transition process; and 3) identify if other candidate genes are involved in this ecologically important morphological plasticity.

518. Identification and functional characterization of fungal membrane proteins related to phosphate fluxes in arbuscular mycorrhizal symbiosis. Y. Ding, M. Harrison. Boyce Thompson Institute, Cornell University, Ithaca, NY.

The arbuscular mycorrhizal (AM) fungi are obligate biotrophs which form an ancient and widespread mutualistic symbiosis with plants. All AM fungi require association with a plant to complete their life cycle, in which nutrient exchange has probably been at the heart of the success of this plant-fungus interaction. The main benefit of the AM symbiosis for the plant is the additional Pi delivered to the root cells by the fungal symbiont. Currently, genes encoding proteins responsible for polyP mechanism and Pi efflux in AM fungi still remain unknown. For characterization of genes from AM fungi involved in Pi delivery process, and presumably important for the functioning of the AM symbiosis, RNA-seq data were generated to obtain a detailed view of AM fungal transcriptomes during associations with symbiotic *Medicago truncatula* mutant plants. Based on the metatranscriptomes, six SPX domain containing membrane proteins were screened out. The expression level of these genes was confirmed by the detection of transcripts using laser microdissection of *M. truncatula* root cortical cells. The protein function was analyzed by yeast complementation test. To further explore the gene function, we implemented a gene silencing approach by using short synthetic antisense oligodeoxynucleotides in AM roots. In the present study, we could show efficient gene silencing effect for selected gene candidates. We predict that this system has great potential to investigate components of polyphosphate metabolism and to determine the mechanisms of symbiotic Pi transport to the plant hosts.

519. Let's play JAZ in the poplar rhizosphere or how mutualistic fungi are coping with jasmonic acid signalling. Claire Veneault-Fourrey[1,2], Sebastian Wittulsky[1,2], Yohann Daguerre[1,2], Annegret Kohler[1,2], Francis Martin[1,2]. 1) INRA, UMR 1136 Interactions Arbres/Microorganismes (IAM), Lab of Excellence ARBRE, Centre INRA de Nancy, Champenoux, France; 2) Université de Lorraine, UMR 1136, Interactions Arbres/Microorganismes (IAM), Faculté des Sciences, Vandoeuvre les Nancy, France.

Essential to forest sustainability are mutualistic ectomycorrhizal interactions (ECM) that exist between soil fungi and tree fine roots. The crosstalk between the partners is fundamental for the timing, establishment and maintenance of beneficial relationships. However, very little is known about how symbiosis is initiated by both partners. The timing of the fungal hyphae entering into the root cortex is expected to be a precisely tuned and tightly controlled mechanism aimed to avoid pathogenic co-colonization. We recently demonstrated that the secreted protein MiSSP7 from the ECM fungus *Laccaria bicolor* is preventing jasmonic-acid (JA)-mediated degradation of *Populus trichocarpa JAZ6* protein (1). This interaction allows fungal accommodation and Hartig net developpement. To further investigate how mutualistic fungus *L. bicolor* counteracts JA-signaling, we aim to characterize the components of the protein complex interacting with PtJAZ6 and triggering the symbiotic signaling pathway.

(1) Plett JM, Daguerre J et al., (2014) A secreted effector protein of Laccaria bicolor is required for symbiosis development', PNAS; 111 (22), 8299–8304.

520. Growth inhibition of fungal species by the Chytridiomycete *Homolaphlyctis polyrhiza*. Steven Ahrendt, Na Jeong, Sapphire Ear, Spencer Swansen, Jason E. Stajich. Dept. of Plant Path. & Microbiology, University of California, Riverside, CA.

Research into new, natural antifungal treatment is increasingly important given growing antifungal drug resistance in pathogenic fungi. Secondary metabolites, compounds not involved in the normal functions of an organism, have proven to be useful sources for antimicrobial compounds. Since most of the research into fungal-derived secondary metabolites focuses on Dikarya fungi due to their diverse product types, the zoosporic fungi represent an understudied group in this regard. These paraphyletic, early diverging lineages, comprising the Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, and Cryptomycota, have a motile life stage and fulfil crucial but poorly explored ecological roles. Therefore, a better understanding of their potential to produce compounds that influence intra-organismal interactions is needed. Comparative genomic analyses have identified a host of degradation enzymes in these fungi, suggesting saprotrophic and sometimes pathogenic associations with other organisms. There are few explored examples of secreted or secondary metabolite molecules produced by any of the zoosporic fungi. *Homolaphlyctis polyrhiza* JEL142 is a non-pathogenic Chytridiomycete and is most closely related to the amphibian pathogen *Batrachochytrium dendrobatidis*. We have demonstrated in preliminary studies that *Hp* can inhibit vegetative hyphal growth of *N. crassa* via an unknown secreted compound, while the sporangia of related Chytridiomycetes do not. Our studies have probed the breadth of fungi whose growth is susceptible to *Hp*, including *A. nidulans*, *T. reesei*, *N. tetrasperma*, *N. discreta*, *C. cinerea*, *R. delmar*, *P. blakesleeanus*, *A. gossypii*, and *S. cerevisiae*. We have found that when media is conditioned by *Hp* growth and subsequent filtration, the bioactivity is stable from -20 to +65 °C, and resists treatment by Proteinase K, suggesting the presence of a secreted, non-protein compound. To better explore *Hp* gene content, we produced an improved genome assembly and annotation, building upon the previous draft genome. An *in silico* prediction of secondary metabolite gene clusters suggested several candidate loci, unique to *Hp* when compared to other chytrids, whose specific functions are currently being elucidated.

521. Identification of candidate *Sclerotinia sclerotiorum* virulence genes utilizing genotype-by-sequencing and association mapping. R. Brueggeman[1], J. Richards[1], C. Qiu[1], L. Aldrich-Wolfe[2], S. Jain[1], J. LeBoldus[1], B. Nelson Jr[1]. 1) Plant Pathology, North Dakota State University, Fargo, ND; 2) Biology Department, Concordia College, Moorhead, MN.

Sclerotinia sclerotiorum is one of the most important broadleaf crop pathogens in the United States. Utilizing a restriction associated DNA genotype-by-sequencing (RAD-GBS) method, we genotyped a diverse natural population of 120 *S. sclerotiorum* isolates collected from 22 hosts and 25 states. A total of 4,544 SNP markers located on 2,788 "sequence tags" were dispersed throughout the 38 Mb genome. This placed a SNP marker every 13.6 kb on average. The genotypic and corresponding phenotypic data collected for lesion length on dry bean, soybean, canola and sunflower for 71 of the isolates was used for association mapping (AM) analysis utilizing JMP genomics software. The significant marker trait associations (MTAs) were located at seventeen loci within the *S. sclerotiorum* genome. The MTAs had remarkable correlations common to both soybean and dry bean at fourteen loci. Five of the loci had strong MTAs (-log10(p) >4) and the remaining twelve loci associated with virulence had weaker associations (-log10(p) >3). Many of the virulence loci identified could be delimited to relatively small genomic regions containing a limited number of candidate virulence genes. We are currently conducting an

RNAseq analysis on infected soybean and dry bean and anticipate that some of the virulence genes underlying the loci identified by the AM analysis will be differentially expressed. The combination of the AM analyses and RNAseq data should identify strong candidate genes providing support for their putative roles in virulence. These candidate genes will be targeted for post-transcriptional gene silencing via stable transformation of siRNA constructs in the pathogen. We will also transform the model host *Arabidopsis* with siRNAs constructs in an attempt to silence the candidate pathogen virulence genes by host-encoded siRNAs via host induced gene silencing (HIGS). Utilizing this new genetic tool we are beginning to uncover some of the secrets of this necrotrophic generalist's virulence genes, which may help in the development of strategies to manage this important pathogen.

522. *Exophiala dermatitidis* as a model for investigating the stress biology of extremophile fungi. Jyothi Kumar, Erin Creasey, Steven D Harris. Center for Plant Science Innovation, University of Nebraska Lincoln, NE.

Extremophiles are broadly recognized as organisms that can live in extreme conditions of temperature, acidity, alkalinity, or salinity. Besides expanding our views on the diversity of life on Earth and perhaps beyond, the study of extremophiles has also provided significant insight into how organisms adapt to stress. Extremophile fungi that primarily colonize exposed rock surfaces, known as "rock inhabiting fungi' (RIFs), were originally discovered in Antarctica, but have since been found throughout temperate habitats. RIFs possess numerous morphological and physiological traits and their oligotrophic lifestyles allow them to thrive in harsh environments that are otherwise detrimental. We propose that the "model" black yeast *Exophiala dermatitidis* can be used to investigate the molecular basis of these traits. The accumulation of pigments, melanin and carotenoids likely play a key role in the stress tolerance of *E. dermatitidis*. In their functional characterization of the GTPases Cdc42 and Rac1, Guo and Szaniszlo (unpublished results) noted that the absence of Cdc42 resulted in apparent loss of carotenoids. We have confirmed this and further shown that Cdc42 mutants fail to induce the carotenoid gene cluster. We are currently using transcriptome sequencing to better define the role of Cdc42 in pigment synthesis and broader stress responses. We have also discovered that *E. dermatitidis* can engage in transient mutualisms with photosynthetic algae to support growth and development. This was accomplished by co-culturing *E. dermatitidis* with the alga *Chlorella sorokiniana* in the presence of light on a minimal medium that otherwise lacked carbon. Preliminary results show that co-cultivation markedly enhances growth, promotes the formation of hyphae, triggers asexual development, and results in stable associations between fungal and algal cells. These results suggest that transient mutualisms may play a significant role in enabling the survival of extremophile fungi in harsh environments.

523. The velvet gene *velA* in *Epichloë festucae* – role in plant symbiosis; plant, nutrition and light-dependent expression; and target genes. Mostafa Rahnama[1], Paul Maclean[2], Richard D. Johnson[3], Richard Gardner[1], Damien J. Fleetwood[4]. 1) School of Biological Sciences, The University of Auckland, Auckland, New Zealand; 2) AgResearch, Hamilton, New Zealand; 3) AgResearch, Palmerston North, New Zealand; 4) Biotelliga Ltd, 3A Symonds St, Auckland, New Zealand.

The velvet gene (*veA* or *velA*) is a key factor in the regulation of fungal development, biosynthesis of secondary metabolites and cell wall metabolism. VelA regulation in *Epichloë festucae* is important for maintaining its mutualistic interaction with the agriculturally important forage perennial ryegrass (*Lolium perenne*). The *velA* gene is upregulated in planta and repressed in most conditions in culture, although the combination of light and potato dextrose medium induces *velA* expression. Infection of perennial ryegrass with Δ*velA E. festucae* mutant causes rapid seedling death in two thirds of infected plants while remaining plants displayed a range in severity of plant interaction phenotypes mostly leading to death after several weeks. Different host responses include cell death, lignin and callose deposition, and reactive oxygen species (ROS) production. We hypothesise these are MAMP-Triggered Immune responses as VelA influences cell wall characteristics of *E. festucae* as shown by altered growth under cell wall stress and mycelial hydrophobicity. Transcriptome analysis of wild type vs. Δ*velA E. festucae* in culture revealed a range of candidate genes potentially involved in plant interaction and cell wall metabolism under VelA regulatory control. Functional analysis of a candidate gene containing two LysM domains is currently underway.

524. *Phytophthora sojae* effector Avr1b can be delivered into soybean cells by heterologous PI3P-binding proteins during infection. Qunqing Wang, Felipe Arredodo, Eli Perez, Brett Tyler. Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR.

Oomycete and fungal pathogens secrete effector proteins that can enter plant cells to modify the physiology of their hosts. A major class of effectors produced by oomycetes contains RXLR motifs that mediate entry of these effectors into plant cells. We previously showed that RXLR effectors can enter host cells in the absence of any pathogen. Furthermore, these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RXLR motif, plus in some cases, C-terminal regions of the protein. Previously we showed that PI3P binding is required for the effectors to enter into host cells when the purified proteins are introduced into root or leaf tissue. Here we show that the RXLR motif of Avr1b is sufficient for cell entry *in vivo*, independent of the C-terminal PI3P-binding residues. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RXLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicated that Avr1b is post-translationally modified.

525. HaCPL2, a cerato-platanin family protein, acts as a protein elicitor in *Heterobasidion annosum*-*Pinus sylvestris* pathosystem. H. Chen, J Quintana Gonzalez, A. Kovalchuk, F. Asiegbu. Department of Forest Sciences, University of Helsinki, Helsinki, Helsinki, Finland.

Heterobasidion annosum sensu stricto (s.s.) is one of the main cause of root and butt rot disease of conifers and the most economically important diseases of forest trees in the Northern Hemisphere. As a necrotrophic pathogen, *H. annosum* has a large secretome which might include a lot of secreted proteins involved in pathogenesis. One of the secreted proteins is Cerato-platanin, a new phytotoxic protein which was first identified in culture filtrates of the Ascomycete Ceratocystis fimbriata f. sp. platani, the causal agent of canker stain disease on the European plane tree *Platanus acerifolia*. In *H. annosum* genome, there are three cerato-platanin homologues (HaCPL1, HaCPL2 HaCPL3) and two of them contain secretion signals. We compared the homologue sequences with the reference protein and HaCPL2 showed the

highest similarity. In the microarray transcript profiling conducted with necrotrophic phase mycelia, only HaCPL2's expression was detected. Additionally, HaCPL2's expression was significantly up-regulated during necrotrophic growth of the fungus on germling roots of g Scots pine seedlings compared to saprotrophic growth on dead pine sapwood and free living growth on malt extract agar. In order to study the eliciting ability, we cloned the HaCPL2 and constructed a recombinant Pichia pastoris over-expression strain X33/pPICZαA/HaCPL2. The recombinant protein was purified with the aid of 6×His tag and confirmed by western blot with antibody against c-myc tag. The recombinant protein secreted to the media appeared to be monomeric, dimeric and polymeric forms which were confirmed by mass spectrometry (MALDI-TOP-TOP). In the preliminary in vitro inoculation experiments with the purified HaCPL2 (a mixture of different forms), it induced necrosis browning on Scots pine seedlings after 48h with seedlings mortality observed after 7 days. It caused loss of leaf turgidity The protein caused loss of leaf turgidity when infiltrated into *Nicotiana tabacum*. The specificity and elicitor ability of HaCPL2 is discussed.

526. Identification of new entomopathogenic fungi in Lebanon and molecular engineering of *Beauveria bassiana*. Sylvain Brun[1], Zakaria Kambris[2]. 1) LIED-UMR 8236, Univ Paris-Diderot, Paris, France; 2) American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut 1107 2020 Lebanon.

Many insects, mosquitoes in particular are vectors of human parasitical diseases like malaria, chikungunya or dengue. These diseases represent a major threat all around the world but especially in tropical and sub-tropical regions. In Lebanon, the Asian tiger mosquito *Aedes albopictus* have been identified about 10 years ago and its population size has greatly increased since then. *Ae. albopictus* is a vector for several viruses including Chikungunya and Dengue. Cases of mosquito-borne diseases are still not common in Lebanon, but the presence of endogenous mosquito vectors together with climatic warming may change the *status quo* in the near future. The classical strategy to contain populations relies on insecticides. However, this leads very often to a rapid selection for physiologically resistant mosquitoes, hence the need for alternative control methods such as the use of entomopathogenic fungi to kill the mosquitoes and/or the manipulation of the symbiotic flora to reduce the mosquito's vectorial capacity. Although these methods are promising little is known on the molecular mechanisms of the host/pathogen interactions. Studies of insect's immune system as well as studies of their fungal pathogens are of main importance in the perspective of controlling the development and the spreading of the parasite. In collaboration with the department of Biology at the American University of Beirut, we are interested in developing new tools to study insect immunity towards fungi. The first part of the project is the isolation of natural enthomopathogenic fungi from Lebanon and to determine the response of insect hosts against them. Two insects models will be studied, *Drosophila* and mosquitoes. The second part of the project consists in modifying the genome of the enthomopathogenic fungus *Beauveria bassiana* in order to study how the deletion of precise genes of the pathogen affect the response of the host.

527. Reduced expression of a glutathione synthase gene (*gsh2*) in *Leptosphaeria maculans* causes loss of pathogenicity on canola (*Brassica napus*). Kylie Chambers, Barbara Howlett, Angela Van de Wouw, Candace Elliott. School of Botany, The University of Melbourne, Melbourne, Australia.

Leptosphaeria maculans causes blackleg, the most serious disease of canola (*Brassica napus*) worldwide. Current approaches to control blackleg are through the use of agronomic techniques such as crop rotations and sowing disease-resistant canola varieties; however, *L. maculans* has overcome major gene resistance in commercially released cultivars. Effective control strategies require knowledge of plant defense and fungal pathogenicity mechanisms. Pathogenicity genes are required for the development of disease and have the potential to be fungicide targets. Agrobacterium T-DNA insertional mutants were generated and screened on wounded cotyledons of *B. napus* cv. Westar for loss of pathogenicity. Seven of 54 mutants screened showed reduced pathogenicity compared to the wild type isolate. Southern blot analyses showed that five of the seven mutants had a single T-DNA insertion, while two had two independent insertions of T-DNA. Locations of T-DNA insertions were determined by Thermal Asymmetric Interlaced PCR (TAIL-PCR) or by Illumina sequencing. The location of the T-DNA insertion in one mutant, UM212, was determined by TAIL-PCR to be between Lema_020000 and Lema_020010. Quantitative reverse transcriptase PCR of both genes indicated that Lema_020010, which encodes a putative glutathione synthase (*gsh2*) was 60% downregulated compared to wild type. Glutathione synthase is the second enzyme involved in the synthesis of glutathione, a ubiquitous tripeptide that plays numerous roles in the cell, including protection against free radicals and reactive oxygen species (ROS) such as hydrogen peroxide. The role of *gsh2* is being investigated in relation to fungal growth, pathogenicity and protection of the fungus against oxidants such as hydrogen peroxide. The mutant is more resistant to hydrogen peroxide than the wildtype isolate is, implying that ROS may be important during infection of canola by *L. maculans*.

528. Manipulation of macrophage biology by the intracellular fungal pathogen *Histoplasma capsulatum*. Bevin English, Young Nam Lee, Dervla Isaac, Charlotte Berkes, Anita Sil. Howard Hughes Medical Institute, Dept. of Microbiology and Immunology, UCSF, San Francisco, CA.

Histoplasma capsulatum (Hc) is a primary fungal pathogen of humans and other mammals. As an intracellular pathogen, Hc is able to subvert the immune function of naïve macrophages and replicate within the phagosome, eventually causing macrophage lysis. However, the mechanism by which Hc causes host-cell death is unknown. Macrophage lysis is dependent upon the secreted protein Cbp1 (calcium binding protein 1, ref 1); we show that *cbp1* mutant yeast are able to grow to high levels within macrophages, but these host cells do not lyse, indicating that high intracellular fungal burden is not sufficient to trigger host-cell death. Because Cbp1 has no known protein domains and only a few orthologs which are relatively unstudied, we undertook two exploratory approaches to begin to elucidate the mechanism by which Cbp1 mediates the interaction between Hc and its host. The first approach is a comprehensive alanine scanning mutagenesis of Cbp1, which enabled us to identify a group of acidic residues at the N terminus that are necessary for macrophage lysis. We are currently assessing the mutant library for other Cbp1 properties, such as calcium binding, which will enable us to either link or uncouple these properties to the ability to trigger host-cell death. The second exploratory approach was transcriptional analysis of infected macrophages, which led to the identification of a set of host genes that are induced during Hc infection in a Cbp1-dependent

manner. Several of these host genes are involved in ER stress and apoptosis, including *Tribbles3* (*TRB3*). Here we show that macrophages deficient for *TRB3* are resistant to Cbp1-mediated lysis. Similarly, macrophages lacking both Bax and Bak, key components of the apoptotic pathway, are also resistant to lysis during Hc infection. These data suggest that Cbp1 induces apoptosis in the host cell, and we are currently investigating host factors that interact with Cbp1 to cause macrophage death.

529. Roles of hydrophilin-like protein in the filamentous fungi *Alternaria brassicicola*. N'GUYEN Guillaume, MARCHI Muriel, DIAS Eva, POCHON Stephanie, CALMES Benoit, BATAILLE-SIMONEAU Nelly, CAMPION Claire, SIMONEAU Philippe, GUILLEMETTE Thomas. SFR Quasav 4207, UMR 1345-IRHS, FungiSem , Faculté des Sciences, 2 bd Lavoisier 49045 Angers cedex 01 France.

During their life cycle, fungi face adverse environmental conditions associated with alterations in water status. Phytopathogenic fungi are faced with this type of stress during the infection process, especially when they colonize seeds. Although these organisms are particularly effective to adapt to these water potential decreases, these coping mechanisms are so far very poorly described, particularly in filamentous fungi. *Alternaria brassicicola* is a seed-borne fungal pathogen responsible for the black spot disease on *Brassicaceae* plants. Alteration of *Brassicaceae* seed quality is one of the most damaging effects of the black spot. Beyond contribution to pathogen dissemination, the presence of the fungus on the seeds compromises seedling germination and survival. To better understand the determinism of fungus transmission to seeds, we previously established a reliable *Arabidopsis*-based pathosystem allowing investigations of *A. brassicicola* transmission to seeds. In particular, we showed that fungal susceptibility to osmotic and water stress influences the seed transmission ability.

Transcriptomic analyzes, carried out under different experimental *in vitro* conditions inducing these types of stress (addition of sorbitol or Poly Ethylene Glycol (PEG)), allowed us to identify additional mechanisms potentially involved in the fungal adaptive responses. In particular, these analyzes revealed a pool of over-expressed genes encoding putative proteins which share physiochemical features typical of hydrophilin-like proteins. We initiated functional studies of some of these hydrophilins by generating respective Knock-Out mutants. Next step of this work is to determine whether these mutants are impaired in their adaptive response to water stress and other types of stress (such as oxidative stress) and whether hydrophilins are involved in pathogenicity. The pathogenic behaviour of mutants will be examined at both vegetative and reproductive plant developmental stages.

530. An endo-Rhamnogalacturonase associated with the necrotrophic pathogen life style of *Heterobasidion irregulare*. Yang Hu[1], Seong-Beom Kim[2], Jun-Hyun Jeon[2], Malin Elfstrand[1], Yong-Hwan Lee[2], Jan Stenlid[1], Åke Olson[1]. 1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Center for Fungal Pathogenesis, Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea.

Heterobasidion annosum sensu lato (s.l.), which causes root and butt rot to conifer, is the most destructive pathogen in boreal and temperate conifer forests of the northern hemisphere. To find efficient ways to control this disease, it is important to understanding the biology of the pathogenicity of the fungus and interactions with its host. A gene encoding endo-rhamnogalacturonase belong to GH28 family was found in QTLs of virulence, and it was highly up regulated during infection compare to other life stages. Phylogeny study of this gene showed that endo-rhamnogalacturonase was lost in most biotrophs and hemibiotrophs investigated. We hypothesis that it could be a PAMP/MAMP which could trigger plant defence in biotroph which results in incompatible reactions, but be beneficial for the necrotroph by promoting necrosis and degrading plant cell walls. The results from characterization of an endo-rhamnogalacturonase from *Heterobasidion* will be presented.

531. Cultural and genomic differentiation between *Sclerotinia sclerotiorum* and *S. trifoliorum*. Teresa Jardini[1], Dan Qiu[1], Weidong Chen[1,2]. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Washington State University, Pullman, WA.

Sclerotinia sclerotiorum and *S. trifoliorum* are closely related species causing devastation on a wide variety of plants. Although the two species appear very similar, *S. sclerotiorum* and *S. trifoliorum* differ widely in host range. *S. trifoliorum* is limited to 40 plant species, mostly cool season legumes, while *S. sclerotiorum* infects over 400 plants, including all host plants of *S. trifoliorum.* To elucidate mechanisms of this host range difference, we examined cultural and transcriptomic differences between the two species. Growth rate and acid production at different temperatures and on media buffered at different pHs were investigated. pH change in cultural media was used as an indicator of acid production. *S. sclerotiorum* grew faster and produced more acid (lower pH) than *S. trifoliorum* at every temperature, except at 5°C. Optimum temperature range for growth was lower (15-20°C) for *S. trifoliorum* than for *S. sclerotiorum* (20-25°C). At each buffered medium pH, *S. sclerotiorum* grew faster and produced more acid (lower pH) than *S. trifoliorum*. Growth and acid production of *S. trifoliorum* was increasingly limited by increasing medium pH, whereas medium pH had little effect on growth of *S. sclerotiorum*. In transcriptomic analysis, approximately 60% of the transcripts were common between the two species, and about 43% of the transcripts were genes with known functions for both species. Among 1411 orthologous contigs, 147 (10%) were highly (> 3 folds) expressed in *S. trifoliorum* than in *S. sclerotiorum*, and 173 (12%) were highly expressed in *S. sclerotiorum* than in *S. trifoliorum*. Approximately 140 transcripts from each species were found in the noncoding regions in the annotated genome of *Sclerotinia sclerotiorum.* Fifteen transcripts in the noncoding region were found in both species. Additionally, differences in expressed genes involved in pH modulation and pathogenesis like oxalate biosynthesis and endopolygalacturonases were detected between the two species. These differences provide biological and molecular bases to further study the host range difference between *S. sclerotiorum* and *S. trifoliorum*.

532. Cell Biology and Biochemical aspects of infection by the root rot fungus *Phymatotrichopsis omnivora*. Prasanna Kankanala, Carolyn A. Young, Kirankumar S. Mysore. Plant Biology, Samuel Roberts Noble Foundation, Ardmore, OK.

Phymatotricum root rot (PRR) also commonly known as cotton root rot / Texas root rot is caused by the ascomycete *Phymatotrichopsis omnivora* (Duggar) Hennebert. Over 2000 species of dicotyledonous plants are susceptible and almost all monoctyledonous plants are known to be tolerant to this fungus under field conditions. In southern Oklahoma and Texas regions this pathogen limits the production of

alfalfa and cotton which are valuable forage and fiber crops respectively. The fungus is a necrotrophic pathogen and is notoriously known for losing virulence under laboratory conditions. We have successfully isolated a virulent strain from an infected field site during summer of 2014 and established pathogen assays both with the model plant,*Medicago truncatula,* as well as a crop plant, *Medicago sativa*. Unfortunately, the early stages of disease occurance are poorly understood due to lack of proper tools. For example, *P. omnivora* is not yet malleable for genetic manipulations. We are using recently developed fluorescent tagged cytoskeleton and organelle markers of *M. truncatula* to better understand the critical aspects of the PRR disease. In addition, we have devloped a minirhizotron system that mimics field like growth conditions to help us better understand the *P. omnivora*-plant interaction. This system will allow us to study the effect of root exudates on PRR disease. A progress on cell biology and biochemical studies to understand *P. omnivora* infection process will be presented.

533. Generation of a ToxA knockout strain of the wheat tan spot pathogen Pyrenophora tritici-repentis. Caroline Moffat, Pao Theen See, Richard Oliver. Curtin UNiversity, Perth, WA, Australia.

The necrotrophic fungal pathogen Pyrenophora tritici-repentis causes tan spot, a major disease of wheat, throughout the world. The proteinaceous effector ToxA is responsible for foliar necrosis on ToxA-sensitive wheat genotypes. The single copy ToxA gene was deleted from a wild-type race 1 P. tritici-repentis isolate via homologous recombination of a knockout construct. Expression of the ToxA transcript was found to be absent in transformants (toxa), as was ToxA protein production in fungal culture filtrates. Plant bioassays were conducted to test transformant pathogenicity. The toxa strains were unable to induce necrosis on ToxAsensitive wheat genotypes. To our knowledge, this is the first demonstration of a targeted gene knockout in P. tritici-repentis. The ability to undertake gene deletions has facilitated the characterization of other pathogenicity effectors of this economically significant necrotroph.

534. Investigating the environmental pressures that shape virulence in the thermally dimorphic fungal pathogen *Histoplasma capsulatum*. Lauren Rodriguez, Anita Sil. Howard Hughes Medical Institute, Dept. of Microbiology and Immunology, University of California San Francisco, San Francisco, Ca 94143-0414.

Temperature is an important signal for pathogens to sense that they are inside of a mammalian host. In the case of thermally dimorphic fungal pathogens, temperature is linked to different morphological states. We study *Histoplasma capsulatum,* which exists in a multicellular mycelial phase in the soil, produces vegetative spores, and transitions to a unicellular yeast phase once inhaled by a mammalian host. Induction of virulence genes is specific to the yeast phase of the fungus, but there is no obvious requirement of host colonization for the survival of the fungus in the environment. How *H. capsulatum* has acquired and maintained its pathogenesis toward mammals remains unclear. Additionally, how thermally dimorphic fungi have evolved to respond to temperature is unknown. I am interested in looking at soil phagocytes such as amoebae as a source of environmental selection for virulence traits. It has been established that the soil amoeba *Acanthamoeba castellanii* readily engulfs *H. capsulatum* yeast, resulting in intracellular growth of the fungus and death of the host cell. These events are similar to what we observe after infection of macrophages. By comparing the interaction between *H. capsulatum* and *A. castellanii* with the interaction between *H. capsulatum* and macrophages, I hope to explore how host signals trigger the transition to a pathogenic state. Preliminary results show that the intracellular environment of *A. castellanii* promotes growth of the pathogenic yeast form independent of temperature. Additionally I will examine whether the morphologic and transcriptional responses of *H. capsulatum* to amoebae are similar to the responses of the fungus to mammalian body temperature. These studies will uncover how fungal responses to host signals have allowed for the selection of virulence traits in thermally dimorphic fungi.

535. What makes the ash dieback fungus *Hymenoscyphus fraxineus* pathogenic? Jan Stenlid[1], Michelle Cleary[2], Mikael Brandström Brandström[1], Malin Elfstrand[1]. 1) Swedish Univ Agr Sci, Dept Mycology & Plant Patholog, Uppsala, Sweden; 2) Swedish Univ Agr Sci, Southern Swedish For Res Centre.

Recently, a new, invasive fungal pathogen (*Hymenoscyphus fraxineus*) affecting common ash (*Fraxinus excelsior*) has emerged as a serious forest health problem in Europe. The fungus is believed to be native in eastern Asia where it is acting as an endophyte in *F. mandschurica*. We have sequenced the genomes of *H. fraxineus* and its European congeneric *H- albidus* and also analysed gene expression following inoculation into ash trees. In our analysis we have identified gene families expanded in these species compared to other members of Helotiales. *H. fraxineus* has an active gene cluster likely to be involved in secondary metabolism that is non-functional in *H. albidus*. Differences in gene expression beween the species when inoculated into ash will be discussed in light of the cantrasting interction outcomes with the host treee.

536. Regulation by RNAi of putative virulence factors in the opportunistic pathogen *Mucor circinelloides*. S. Torres-Martinez[1], F.E. Nicolás[1], A. Vila[1], A. López-Muñoz[2], M.A. Hernández-Oñate[3,4], A. Herrera-Estrella[3], V. Mulero[2], C. Pérez-Arques[1], M.I. Navarro-Mendoza[1], R.M. Ruiz-Vázquez[1], V. Garre[1]. 1) Dept Gen & Microbiol, Univ Murcia, Murcia, Spain; 2) Dept Cell Biol & Histol, Univ Murcia, Murcia, Spain; 3) Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV-IPN, Irapuato, Guanajuato, México; 4) Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, AC, Hermosillo, Sonora, México.

The basal fungus *Mucor circinelloides* is a good model organism for studying different molecular processes in the fungal kingdom, mainly due to the availability of a number of molecular tools and to its evolutionary distance from other fungal models. Recently, *Mucor* is attracting special attention as a causal agent of mucormycosis, an emerging fungal infection, not very common but often lethal. *Mucor* is one of the first fungi in which several classes of endogenous small RNAs (esRNAs) with regulatory functions have been identified. Many of these esRNAs derive from exons (ex-siRNAs) and are produced by different RNAi pathways. Since these RNAi pathways play relevant roles in the regulation of physiological and developmental processes in *Mucor,* we have explored the possible role of the ex-siRNAs and the RNAi machinery in *Mucor* pathogenesis. For that, different strategies have been developed, including (i) transcriptomic analysis by RNA-seq to characterize the mRNA profiles shown by *Mucor* mutants in the main RNAi genes, (ii) identification of esRNAs and mRNAs

differentially accumulated in pathogenic and non-pathogenic strains of *Mucor* and, (iii) the use of zebrafish as an animal model for host-pathogen interaction to identify differentially expressed ex-siRNAs and mRNAs, both in the host and in the pathogen, as a consequence of the infection with pathogenic and non-pathogenic strains. Results from the analysis of the extensive datasheet obtained signal genes regulated by esRNAs and the RNAi machinery as candidates to be considered possible virulence factors involved in the pathogenicity of *Mucor*.

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537. Pathogenicity screening of Finnish isolates of the conifer pathogen *Heterobasidion annosum s.s* and *Heterobasidion parviporum* for identification of pathogenicity-specific genomic regions. Z. Zeng[1], H. Sun[1], T. Raffaello[1], E. Jaber[1], K. Korhonen[2], F. Asiegbu[1]. 1) Department of Forest Sciences, University of Helsinki, Latokartanonkarri 7, 00014, Helsinki, Finland; 2) Finnish Forest Research Institute (METLA, Finland).

The Nordic countries have extensive forests, mostly dominated by conifer trees. Most conifer trees are susceptible to infection by the root and butt rot fungus *Heterobasidion annosum*, which is one of the most destructive disease in the northern temperate regions of the world. The combined direct and indirect economic losses caused by *H. annosum* infection approximate to 50 - 100 million euros yearly in Finland alone. This fungus has three Eurasian and two North American intersterility groups based on their main host preferences. The Eurasian P intersterility group (*H. annosum s.s*) and S group (*H. parviporum*) attacking Scots pine and Norway spruce respectively are prevalent in to Finnish forestry. Therefore, the purpose of this work was to identify the pathogenicity-specific genomic regions in these two intersterility groups that distinguish them as the most devastating forest pathogen. In our project, 17 isolates of the P group and 24 isolates of the S group were obtained from infected tree tissues or fruiting bodies in Finnish forests from 16 and 21 geographic locations respectively. The pathogenicity of these isolates was screened by infecting either 2- 3 weeks old seedlings or 3- to 5-year-old Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) plants. Pathogenicity level was determined using the extent of mortality or necrosis level of the seedlings post-inoculation as the criteria. The four isolates classified as having the highest and the lowest pathogenicity in P-type and S-type were selected. Homokaryotic mycelia of the 4 selected isolates were obtained and genomic DNAs were isolated to be used for *de novo* genome sequencing. Genome assembly, annotation and comparative genomic analysis will be carried out to find out the pathogenicity-specific regions in both the P group and S group of *H.annosum*..

538. Analyses of xylem sap proteins in susceptible and resistant tomato-*Verticillium dahliae* interactions. Xiaoping Hu[1], Steven J. Klosterman[2], Suraj Gurung[3], Dylan P. G. Short[3], Monica Britton[4], Blythe Durbin-Johnson[4], Brett Phinney[4], Michelle Salemi[4], Krishna V. Subbarao[3]. 1) Northwest Agriculture and Forestry University, Yangling, China; 2) USDA-ARS, Salinas, CA; 3) University of California, Davis, Salinas, CA; 4) Genome Center and Bioinformatics Core Facility, University of California, Davis, Davis, CA.

Verticillium dahliae is a soilborne fungus that infects plant roots, invades the water conducting xylem tissue, and causes characteristic leaf wilting and other symptoms. In this study, tomato plant xylem sap was extracted from both *V. dahliae* susceptible and resistant tomato following inoculation with *V. dahliae*, and the respective proteomes were analyzed for proteins expressed in the susceptible *Verticillium*-tomato interactions and a resistant interaction. Compared to the mock-inoculated control, there was a significant overrepresentation of the gene ontology term response to biotic stimulus associated with the xylem sap proteins of inoculated plants, while the resistant (inoculated) interaction also displayed significant overrepresentation of the term lignin biosynthetic process. Among the top 20 most abundantly expressed proteins across all interactions, including the mock controls, analyses of the total peptide matches revealed increased expression of a serine endopeptidase and a simultaneous decrease in the expression of a tomato endochitinase in the xylem sap of the susceptible *V. dahliae*-tomato interaction. Identifications of xylem sap proteins expressed differentially in susceptible and resistant *V. dahliae*-plant interactions are anticipated to reveal insight into both fungal proteins important for virulence and plant proteins that mediate defenses.

539. Dual RNA-Seq of the hemibiotrophic pathogen *Setosphaeria turcica* and its host, maize. T. Wiesner-Hanks[1], B. Condon[1], S. Saha[1,2], D. Wu[1], S. Mideros[1], R. Nelson[1], B.G. Turgeon[1], Joint Genome Institute. 1) School of Integrative Plant Science, Cornell University, Ithaca, NY; 2) Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY.

Dual RNA-seq is a promising tool to characterize the *in planta* interplay between pathogen and host transcriptomes. We sequenced the transcriptomes of *Setosphaeria turcica*, a hemibiotrophic fungal pathogen, and its host maize (*Zea mays*), at several stages during pathogenesis. Leaves of susceptible maize variety B73 were inoculated with a *S. turcica* race 23N strain, and RNA from leaf tissue was sampled at four time points after inoculation and mapped to the *S. turcica* and maize genomes. Transcripts were tested for differential expression using the DESeq, edgeR , and DESeq2 methods. Networks of genes with similar expression profiles were constructed using weighted gene correlation network analysis (WGCNA). In both *S. turcica* and maize, the transcriptional profiles undergo a major shift between 5 and 7 days, likely reflecting the transition of *S. turcica* into its necrotrophic phase. A hybrid polyketide synthetase/nonribosomal peptide synthetase gene, confirmed previously to be necessary for virulence by gene knockout, was the second most significantly upregulated transcript in *S. turcica* overall. Many regions of the maize genome implicated in resistance to *S. turcica*, by mapping and genome-wide association, were sites of enriched differential expression during pathogenesis. Forthcoming RNA-seq datasets will explore the transcriptome-wide implications of the *Ht2* resistance gene in maize and the counterpart effector in *S. turcica*. *Ht2* +/- isolines of maize were inoculated with race 1 (avirulent on *Ht2*+) and race 23N (virulent on *Ht2*+) isolates of *S. turcica*, yielding dual transcriptomes from four unique combinations of host and pathogen.

540. Investigating secondary metabolism in *Zymoseptoria tritici*. Solaf Ali, Andy Bailey. Life Sciences Building, 24 Tyndall Avenue, University of Bristol, Bristol, BS8 1TQ United Kingdom.

One of the most serious fungal diseases of wheat is Septoria Tritici Blotch caused by *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*). The progression of this disease is typified by an asymptomatic latent phase of a week or more, followed by a rapid onset on

host-cell necrosis. This sudden cell-death has been speculated to be caused as a result of toxins made by the fungus. Fungally derived toxins are often secondary metabolites, with the main classes being polyketides (PKs) non-ribosomal peptides (NRPs) and terpenes. Genome analysis of *Z. tritici* identified nine PKS and two hybrid PKS-NRPS pathways in addition to the NRPS thought to be responsible for siderophore biosynthesis for iron acquisition. Promoter:GFP fusions were made for all of these candidate toxin synthase genes and their expression followed *in vitro* and though the disease cycle by confocal microscopy, however only pPKS3 gave any apparent GFP expression. Disruption of PKS3 did not generate any mutant phenotype. All PKS and NRPS enzymes require activation with phosphopantetheine and disruption of the Phosphopantetheine transferase (PPTase) has been reported to eliminate infection by *Z. tritici.* In fungi, PPTase is needed not only for the PKS and NRPS activation, but also both lysine and siderophore synthesis. The *Z. tritici* aminoadipate reductase (Lys2) gene was disrupted and as expected the *ZtΔlys2* mutant was auxotrophic to lysine. Furthermore, the pathogenicity testing of *ZtΔlys2* on wheat showed a significant reduction in the pycnidia and spore number compared with a wild type. In a parallel experiment, the ornithine N hydroxylase (SidA) was disrupted to prevent hydroxamate siderophore synthesis. The resulting *ZtΔSidA* mutant was auxotrophic for iron, more sensitive to the oxidative stress compared with wild type. Furthermore, ZtΔSidA produced less pycnidia and spores and showed slower (but not abolished) plant infectivity. Therefore whilst pantetheine-based secondary metabolism is essential to *Z. tritici*, we cannot determine whether this is solely due to the lysine and iron effects, or whether there may also be secondary metabolism effects.

541. Secondary metabolite hunting in the wheat pathogen *Parastagonospora nodorum*. Mariano Jordi Muria Gonzalez, Peter Solomon, Susan Breen. Plant Sciences, RSB, Australian National University, Australia.

The dothideomycete *Parastagonospora nodorum* is a pathogen of wheat and the cause of significant yield losses globally. Recent studies have demonstrated that *P. nodorum* secretes effector proteins during infection that interact with dominant host susceptibility genes leading to necrosis and disease. Whilst the requirement of these proteinaceous effectors is clear, the role of *P. nodorum* secondary metabolites in this interaction remains a mystery. Despite the fact that *P. nodorum* harbours more than 40 secondary metabolite gene clusters, we know little of neither their identity nor function.

In this project, we explore the chemical interchange that occurs between *P. nodorum* and other organisms that the pathogen may encounter during its life cycle. A mix of traditional natural product and mass spectrometry-based approaches has been exploited to identify new secondary metabolites synthesized by *P. nodorum.* During this study, small molecules not previously reported for *P. nodorum* have been identified and their phytotoxicity assessed. Furthermore, we have also screened these small molecules against other possible pathogens and saprophytes that *P. nodorum* may encounter during its lifecycle. To complement the pathogen side of the interaction, we have also begun assessing the role of host secondary metabolites during disease and identified several novel antifungal activities in wheat that are modulated by *P. nodorum* infection.

Since secondary metabolites have been related to ecological adaptation, understanding their bio-ecological role, will not just help us to better understand the lifestyle of this pathogen, but may also highlight critical stages in the fungal disease cycle that can be targeted to tackle this devastating disease.

542. A *Setosphaeria turcica* secondary metabolite effector prompts a resistance response on *Ht1* maize. Dongliang Wu[1], Santiago Mideros[1], Tyr Wiesner-Hanks[2], Rebecca Nelson[1,2], B. Gillian Turgeon[1]. 1) Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY; 2) Plant Breeding and Genetics Section, School of Integrative Plant Science,Cornell University, Ithaca, NY.

Setosphaeria turcica (*Exserohilum turcicum*) is the causal agent of Northern Leaf Blight of maize, one of the most important maize foliar diseases. *S. turcica* is a hemibiotrophic pathogen and several races have been described based on the symptoms they cause on a panel of maize lines carrying the 'major resistance' genes, *Ht1, Ht2, Ht3,* and *HtN*. By definition, maize carrying *e.g., Ht1* is susceptible to *S. turcica* race 1. Our recent evidence suggests that a gene encoding a hybrid polyketide synthase:nonribosomal peptide synthetase (PKS:NRPS) enzyme for biosynthesis of a secondary metabolite in *S. turcica* race 23N sequenced strain 28A is critically important for the specific resistance interaction of this race on *Ht1* maize. Thus, mechanism of action is similar to the (hemi)biotrophic microbial effector protein-plant resistance protein model, but different in that a secondary metabolite, not a protein, acts as a fungal effector. Genotyping by sequencing and analysis of >200 progeny of a *S. turcica* race 23N by race 1 cross and concomitant phenotyping on *Ht1* maize identified a SNP in the same *PKS*:*NPS* gene, that segregates perfectly with susceptible/resistant phenotypes. The *S. turcica PKS:NPS* gene is highly expressed *in planta* in the resistant interaction. On the maize side, qRT-PCR of maize inoculated with *S. turcica* wild type race 23N and the mutant demonstrate that both salicylic acid (SA) and jasmonic acid (JA) signaling are involved. Thus, a fungal small molecule associates in a specific manner with a plant resistance protein to prompt resistance. This is an underappreciated, emerging area of fungal-plant investigation.

543. Sensing and signalling through the cAMP/PKA pathway is crucial for maintaining a mutualistic symbiotic interaction between *Epichloë festucae* and *Lolium perenne*. Alexander Bisson, Carla Eaton, Barry Scott. Institute of Fundamental Science, Massey University, Palmerston North, New Zealand.

Growth of the fungal endophyte *Epichloë festucae* in mutualistic symbiotic association with *Lolium perenne* (perennial ryegrass) is highly regulated and synchronised with the growth of the host plant. To maintain this pattern of fungal growth *in planta*, specific signalling between the mycosymbiont and its host grass is required. To sense the extracellular environment and respond to changes, filamentous fungi rely on G protein-coupled receptors (GPCRs), which transmit signals predominantly via heterotrimeric G proteins to downstream pathways such as the cAMP/Protein Kinase A (PKA) and MAP kinase-signalling pathways. In phytopathogenic fungi G protein signalling and the

associated cAMP/PKA pathways are often essential for a normal host interaction. Signal transduction by cAMP activation of PKA is finely balanced by attenuation of the signal through a regulatory feedback loop controlled by 3'-5'-cyclic nucleotide phosphodiesterases (PDE). In a comprehensive bioinformatics analysis of the *E. festucae* genome we identified genes encoding three cAMP-receptor-like GPCRs (*gpr1a*, *gpr1b* and *gpr2*), among numerous other genes putatively involved in G-protein and cAMP/PKA signalling. Plants infected with an *E. festucae Δgpr1b* mutant had a severe host interaction phenotype whereas plants infected with *Δgpr2* grew as well as those infected with wild-type. Two *E. festucae* genes encoding putative PDEs involved in regulation of the cAMP-mediated signal were also identified. Deletion of *pdeH*, a gene encoding a high affinity PDE, had a severe host interaction phenotype. Hyphae of *ΔpdeH* were more abundant in the intercellular space and showed extensive colonisation of the vascular bundles and leaf surfaces. In contrast, deletion of *pdeL*, a gene encoding a low affinity PDE, had no effect on the host whole plant and cellular interaction phenotypes. These results show that signalling through Gpr1b and the cAMP/PKA pathway is important in regulating the mutualistic symbiotic interaction between *E. festucae* and *L. perenne*.

544. *Trichoderma* secondary metabolism and its relation to plant growth promotion. Maria Fernanda Nieto Jacobo[1], Fatima Berenice Salazar-Badillo[1,2], Mark Braithwaite[1], Dianne Nguyen[1], Michael Rostas[1], Jorge Teodoro de Souza[1], Johanna Steyaert[1], Juan Francisco Jimenez-Bremont[2], Alison Stewart[1,3], Artemio Mendoza[1]. 1) Bio-Protection Reserch Centre, Lincoln University, Lincoln, Lincoln, New Zealand; 2) San Luis Potosi Institute of Scientific Research and Technology, San Luis Potosi, Mexico; 3) Marrone Bio Innovations, 2121 Second St, Davis, California 95618, USA.

Endophytic *Trichoderma* strains can improve plant growth, confer disease resistance and abiotic stress tolerance, and at the same time, obtain nutrients from their host plants without causing apparent disease symptoms. The continuation of a symbiotic relationship between endophytes and their respective hosts requires constant communication between the organisms. We have been investigating the role of the fungal-derived phytohormone indole acetic acid (IAA) and volatile organic compounds (VOCs) in the signalling dialogue between *Trichoderma* and the model plant *Arabidopsis thaliana*. A range of *Trichoderma* strains, selected for their ability to enhance root production in greenhouse experiments, were tested for IAA production in synthetic medium. Significant differences were observed in the ability to synthetize IAA between strains, and it was found to be medium dependent. Most of the *Trichoderma* strains induced anthocyanin production in the leaves, which may be an indication of stress. No significant differences were observed in most of *Trichoderma* strains in their abilities to induce *A. thaliana* root growth on Murashige & Skoog plates. In a separate bioassay conducted in gamma radiated soil, contrasting results were observed. While four strains significantly enhanced shoot weight, two had no effect on shoot weight and one strain negatively impacted total plant fitness, reducing growth by 50% compared to the untreated control. Thus, we suggest that although IAA could have an important role in the plant growth promotion, additional signals need to be considered in this process such as VOCs. Using a Petri dish split system, we demonstrated that VOCs produced by *Trichoderma* have an impact on plant fitness. The VOCs profiles of different strains will be discussed.

545. Enhancing the Transmission and Viability of Beneficial *Epichloë* Endophytes in Seed. Milan Gagic, Syd Easton, Marty Faville, Richard Johnson, Debbie Hudson, Linda Johnson, Christine Voisey. AgResearch, Palmerston North, New Zealand.

Little is currently known about why some *Epichloë*-grass combinations transmit endophyte successfully into seed and others do not. There is evidence that incompatibility between the endophyte and the host affects the transmission rate, but the underlying mechanisms are not known. This lack of knowledge has delayed the commercial development of some endophyte strains as they fail to meet minimum infection criteria. Infection of seed may fail at three stages of the symbiotic lifecycle; i. colonisation of apical/axillary meristems. ii. colonisation of floral meristems, iii. hyphae fail to remain viable during seed storage. The objective of our study is to identify markers underpining successful endophyte transmission and viability via an integrated approach involving genetic, morphological, and transcriptomics studies. Five independent ryegrass populations infected with the *E. festucae* strain AR37 were used to develop a panel of host genotypes that vary with respect to transmission and viability of endophyte in seed. Plants within each population of endophyte - infected perennial ryegrass have been inter-crossed and the endophyte transmission efficiency scored within 100 progeny seedlings. There were significant differences in endophyte infection between the five populations. For populations 1 to 3, infection frequency was near or above 90%. Populations 4 and 5 had a higher range of infection frequency, 69% and 70% respectively. All populations had individuals with very low endophyte transmission. Replicate plants from two populations have been planted in contrasting environmental locations to validate this data and to determine the impact of genotype x environment on endophyte transmission. In another project, the five populations are currently being genotyped using genotyping by sequencing (GBS) technology. GBS markers statistically associated with endophyte transmission will be identified and mapped to an assembled perennial ryegrass genome. The multifaceted approach to this question will enable us to verify the role of any GBS-linked candidate genes in the endophyte transmission pathway with genes identified from a concurrent transcriptomics experiment. We present the data obtained so far and future prospects.

546. Identification of a novel secondary metabolism gene cluster essential for the *Epichloë festucae-Lolium perenne* symbiosis. Daniel Berry[1], Paul Dijkwel[1], Carolyn Young[2], Barry Scott[1]. 1) Massey University, Palmerston North, New Zealand; 2) The Samuel Roberts Noble Foundation, Ardmore, OK.

Epichloë festucae is a fungal endophyte of *Lolium* and *Festuca* cool season grasses, living within the intercellular spaces of the host in a mutualistic symbiosis. RNAseq analysis of differentially expressed fungal genes between *L. perenne* (perennial ryegrass) plants infected with *E. festucae* wild-type and the stress activated MAP kinase mutant Δ*sakA*, which forms a symbiosis-defective association, identified a putative symbiotically regulated gene cluster[1]. One gene from this cluster, *irlA*, was also down regulated in *L. perenne* infected with the Δ*noxA* or Δ*proA* symbiosis-defective *E. festucae* mutants (Eaton *et al*. unpublished results), implying a core symbiotic function for this gene. The encoded protein IrlA has significant sequence identity and predicted secondary structure to plant isoflavone reductases, indicating a role in metabolite synthesis. Δ*irlA* mutants had a symbiosis-defective phenotype very similar to Δ*noxA* and Δ*proA* associations. Synteny analysis of the *irlA* locus across seven *Clavicipitaceae* family genera identified a five-gene cluster found only within

the *Epichloë spp.* and *Aciculosporium take*, an endophyte of bamboo. No significant homologs of these cluster genes were identified outside of *Epichloë* and *Aciculosporium*, except in the distantly related *Glarea lozoyensis* (Helotiales), which contained the full five-gene cluster. The extent of DNA sequence conservation between the homologous gene clusters in *Epichloë, Aciculosporium and Glarea* indicates this cluster may have originated via horizontal gene transfer. Putative functions for the other proteins encoded by the *irlA* gene cluster include a core single-module non-ribosomal peptide synthetase, a PLP-dependent transferase, an FAD-dependent oxidoreductase and a MFS transporter. We propose these proteins catalyse the synthesis and transport of a novel secondary metabolite required for regulating the symbiotic interaction between *L. perenne* and *E. festucae*. (1) Eaton et al. (2010), Plant Phys. 153: 1780-1794.

547. Two *Epichloë festucae* genes encoding putative membrane associated proteins are required for cell-cell fusion and maintenance of a mutualistic symbiotic interaction with *Lolium perenne*. K. Green[1], Y. Becker[1], H. Lalucque[2], P. Silar[2], B. Scott[1]. 1) Institute of Fundamental Science, Massey University, Palmerston North, Manawatu, New Zealand; 2) Institut de Génétique et Microbiologie, University of Paris, France.

Epichloë festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with *Lolium perenne*. From a series of forward and reverse genetic screens we have shown that single gene disruptions of *noxA, noxR* and *racA*[1], encoding components of the NADPH oxidase complex, *proA*[2], encoding the transcription factor ProA, and *mkkB* and *mpkA*[3], encoding the MAPK kinase and MAP kinase components of the cell wall integrity signalling pathway, leads to a severe host interaction phenotype and a loss of hyphal fusion in culture. Interestingly, homologues of these genes in *N. crassa, S. macrospora* and *P. anserina* are required for cell-cell fusion and sexual fruiting body maturation, thereby establishing a link between self signalling and hyphal network formation during *E. festucae* symbiosis. The aim of this project was to test if *E. festucae* homologues of two recently identified *P. anserina* self signalling genes[4], *IDC2* (*N. crassa Ham-7*) and *IDC3*, which we have named *symB* and *symC*, are also required for symbiosis and hyphal network formation. In contrast to wild-type *ΔsymB* and *ΔsymC* mutants are totally deficient in cell-cell fusion, hyperconidiate and form intra-hyphal hyphae in culture. Within *L. perenne, ΔsymB* and *ΔsymC* mutants cause stunting, hypertillering, premature leaf senescence, vascular bundle colonization and a loss of hyphal network formation. These phenotypes are identical to those observed with *noxA, proA, mkkB* and *mpkA* suggesting that SymB and SymC may interact to form a membrane associated sensor complex to regulate cell-cell fusion and hyphal network development via the cell wall integrity MAP kinase pathway. (1) Tanaka et al. (2006). Plant Cell 18: 1052-1066; (2) Tanaka et al. (2013) Mol Microbiol 90: 551-568; (3) Becker et al. (2015). Mol Microbe-Plant Interact. doi:10.1094/MPMI-06-14-0183-R; (4) Lalucque et al. (2013). 27th Fungal Genetics Conference, Asilomar. p. 159.

548. Gene expression patterns of *Trichoderma virens* during interaction with plant roots. Benjamin Horwitz[1], Netta Li Lamdan[1], Maria E. Morán-Diez[2,4], Naomi Trushina[1], Lea Rosenfelder[1], Prasun K. Mukherjee[2,3], Tamar Ziv[1], Charles M. Kenerley[2]. 1) Technion - IIT, Haifa, Israel; 2) Texas A&M University; 3) BARC, Mumbai, India; 4) Lincoln University, New Zealand.

To test the hypothesis that gene expression of *Trichoderma virens* in its beneficial interaction with plants depends on the host, we co-cultured *T. virens* with maize or tomato in a hydroponic system. The transcriptomes for *T. virens* alone and with tomato or maize roots were compared by hybridization on oligonucleotide microarrays of 11645 unique probes designed from the predicted protein-coding gene models [1]. The transcript levels of 210 genes were modulated by interaction with roots. Of the genes up-regulated on either or both hosts, 35 differed significantly in their expression between maize and tomato. Ten of these had higher expression level in co-culture with tomato roots compared with maize, while 25 were expressed more strongly in co-culture with maize. Among the differentially expressed genes, glycoside hydrolases and transporters were highly represented and suggest an important factor in the metabolism of host cell walls during colonization of the outer root layers. Host-specific gene expression may contribute to the ability of *T. virens* to colonize the roots of a wide range of plant species. Choosing the maize interaction as a model, we compared the secretomes of axenic *T. virens* cultures with *T. virens* – maize co-cultures, using label-free proteomics. Seventeen glycosyl hydrolases were up-regulated and ten down-regulated, at the protein level. Surprisingly, 13 small secreted proteins (SSPs) were down-regulated, essentially independent of their transcript levels, suggesting a protein-specific level of regulation. We are testing the significance of this by construction of knockout mutants in some of the corresponding SSP genes.

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[1] Kubicek et al. (2011) Genome Biology 12(4):R40.

549. *Epichloë* fungal endophytes and the formation of synthetic symbioses in Hordeeae (=Triticeae) grasses. Richard Johnson[1], Marty Faville[1], Milan Gagic[1], Paul Maclean[2], Wayne Simpson[1], Linda Johnson[1]. 1) Forage Improvement, AgResearch Grasslands, Palmerston North, New Zealand; 2) Knowledge & Analytics, AgResearch Ruakura, Hamilton, New Zealand.

Epichloë (formerly including the genera *Epichloë* and *Neotyphodium*) are grass-colonising fungi, belonging to the family Clavicipitacea, that infect grasses within the subfamily Pooideae including some within the tribe Hordeeae. These fungi produce a number of secondary metabolites in their host plants that can be of benefit in agricultural systems, as afforded by *Epichloë* endophytes in Poeae grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*). There have been no accounts of modern domesticated Hordeeae hosting *Epichloë* endophytes, but there have been reports in *Elymus*, *Hordeum* and other wild grasses within the tribe. We have screened over 1000 seed accessions worldwide of *Elymus* and *Hordeum* and have characterised over 100 genetically distinct *Epichloë* strains from these wild populations. Inoculation of modern cereals such as wheat and rye indicates that a range of host-endophyte compatibility outcomes are possible, ranging from incompatible (stunted plants) to fully compatible symbioses. Further to this we have demonstrated that both endophyte strain and host genotype influence the compatibility outcome. To understand the molecular determinants

of incompatible versus compatible host-endophyte associations we have performed transcriptomics experiments (RNA-Seq) on a range of endophyte-host combinations with different compatibility outcomes. A number of host defence related pathways have been identified during incompatible associations and fungal differentially expressed genes are enriched for small secreted proteins (putative effectors). Bioinformatics analysis of several different *Epichloë* genomes has shown that the number of these putative effectors differs significantly between strains with some being unique to a particular strain. Knowledge of these host and endophyte factors will guide us in our aim to create fully functional cereal-endophyte associations.

550. Fungal-bacterial interactions are mediated by fungal lipid signaling and a common set of bacterial factors. O. Lastovetsky[1], S. Mondo[2], T. Pawlowska[2]. 1) Graduate Field of Microbiology; 2) School of Integrative Plant Science, Cornell University, Ithaca, NY.

Fungal-bacterial symbioses are an emergent field of study, and currently little is known about how they are established and maintained. In particular, the genetic basis for interaction between fungi and bacteria is poorly understood. We employ the association between the fungus *Rhizopus microsporus* and the endosymbiotic bacterium *Burkholderia* sp. as a model to identify genes involved in fungal-bacterial interaction and symbiosis. Within the *R. microsporus* species there are isolates that harbor endobacteria (*host*) and there are naturally endobacteria-free isolates (*non-host*). We analyzed fungal and bacterial gene expression during compatible (host with endobacteria) and incompatible (non-host with endobacteria) interactions. This analysis identified dramatic transcriptional changes in the host in response to its bacterial endosymbiont (>750 genes), as compared to the non-host (48 genes). Notably, genes involved in receptor signaling, actin rearrangement and lipid metabolism were overexpressed in the host. In both fungi, responses to bacteria converged on the production of two lipid signaling molecules – diacylglycerol (DAG) and phosphatidic acid (PA). While DAG and PA are interconvertible in eukaryotic cells, they control different pathways. Gene expression in the host fungus pointed to maintaining higher levels of PA over DAG, and the opposite occurred in the non-host. We speculate that maintaining higher levels of one versus the other controls the establishment of symbiosis. Analysis of bacterial transcriptomes showed that bacteria responded to both host and non-host fungi in very similar ways. This allowed for the identification of a common set of mechanisms that bacteria use for interaction with fungi. These included Type 3 Secretion System and its effectors, capsular polysaccharides and a 2-component regulatory system. Interestingly, these mechanisms are also known to be important for the interaction between bacteria and other eukaryotic hosts such as plants and animals. We thus showed that bacteria possess a common set of mechanisms for interaction with plants, animals and fungi.

551. The endophytic symbiont *Epichloë festucae* establishes an epiphyllous network on the surface of *Lolium perenne* leaves by development of an expressorium, an appressorium-like leaf exit structure. Matthias Becker, Yvonne Becker, Kimberly Green, Barry Scott. Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand.

The biotrophic fungus *E. festucae* colonizes the intercellular spaces of the aerial tissues of Festua and Lolium grasses, including leaf primordia, sheath and blade tissue. Besides forming an endophytic hyphal network, *E. festucae* also grows as an epiphyte but the mechanism whereby it establishes a network on the surface of the leaf is not known. Using a combination of confocal laser scanning (CLS)-, scanning electron- and transmission electron- microscopy we have identified a novel structure, which we have named an expressorium to distinguish it from the appressorium used by plant pathogens to enter plants, that allows endophytic hyphae to exit to the leaf surface. The expressorium is a swollen hyphal compartment, often delimited by two septa, that develops just below the cuticle after the hyphae have passed through the epidermis. CLSM analysis of aniline blue/WGA-AF488 co-stained samples revealed a major remodelling of the fungal cell wall following exit from the leaf. Only the septa of endophytic hyphae fluoresce with WGA-AF488 whereas the entire cell wall of epiphytic hyphae fluoresce, suggesting cell wall chitin is either absent or masked in the former but not the latter; results consistent with the need to avoid a host defence response. Given both the Nox1 and Nox2 NADPH oxidase complexes are required for the differentiation of appressoria in *M. oryzae*, as are NoxB and NoxR in *B. cinerea* we tested the effects of *noxA, noxB, noxAB* and *noxR* mutants on the development of expressoria in *E. festucae*. All of these mutants showed defects in the formation of expressoria and instead formed abundant hyperbranched sub-cuticular hyphae. These hyphae eventually breach the cuticle to form epiphyllous pseudo-networks of hyphae that fail to undergo cell-cell fusion (*noxA, noxAB, noxR*) but hyperconidiate (*noxA, noxAB*); phenotypes previously reported for these mutants in axenic culture. These results highlight the importance of the expressorium for *E. festucae* to transition from endophytic to epiphytic growth and the major cell wall remodelling that accompanies these distinct physiological states.

552. Identification and characterization of a nuclear protein, NsiA, essential for hyphal fusion and symbiotic infection of endophytic fungus *Epichloë festucae*. Y. Ozaki[1], A. Tanaka[1], S. kameoka[1], A. Okamura[1], Y. Kayano[1], S. Saikia[2], B. Scott[2], D. Takemoto[1]. 1) The Graduate School of BioAgricultural Sciences, Nagoya University, Nagoya, Japan; 2) Institute of Fundamental Sciences and National Centre for BioProtection, Massey University, Palmerston North, New Zealand.

The endophytic fungus *Epichloë festucae* systemically colonize the intercellular spaces of *Lolium* and *Festuca* cool season grasses to establish a mutualistic symbiotic association. Previous studies have established that *E. festucae* and related endophytes confer bioprotective benefits to their host plants. A screen to identify symbiotic genes isolated a fungal mutant FR405 that altered the interaction from mutualistic to antagonistic. Perennial ryegrass infected with this mutant become severely stunted, show hypertillering and premature senescence, as previously isolated *noxA* (a NADPH oxidase) and ProA (Zn(II)2Cys6 transcription factor) mutants. FR405 has a plasmid insertion in the coding region of uncharacterized A (Alanine), G (Glycine), S (Serine) and P (Proline)-rich protein. GFP fusion of this protein was localized to nuclei, thus we designated this gene *NsiA* for nuclear protein for symbiotic infection. In axenic culture, *noxA* and *proA* mutants lost the ability to form vegetative hyphal fusions and showed significant increase of conidiation. *nsiA* mutants also showed no hyphal fusion as *noxA* and *proA* mutants, although conidiation was normal as wild type. These results indicated that NsiA is involved in similar processes as NoxA and ProA, but play roles downstream of NoxA and ProA in the signal transduction for the regulation of symbiotic hyphal growth. By yeast two-hybrid assay, it was shown than NsiA can interact with the homeodomain of Ste12, a C_2H_2 zinc finger transcription factor. RNAseq analysis revealed that the expression of several genes required for hyphal fusion in *Neurospora*

crassa, *Adv-1*, *Ham8* and *Ham6*/*Pro41*, are down-regulated in the *nsiA* mutant. *E. festucae ham8* and *pro41* mutants lost the abilities to establish symbiotic infection with the host plant as well as that to undergo hyphal cell fusion. These results indicated that NsiA regulate the activity of transcription factor(s) to control hyphal fusion and symbiotic growth of *E. festucae*..

553. Acting under stress – how ectomycorrhizal partners counter metal stress. Manuela Östreicher, Katrin Krause, Erika Kothe. Microbial Communication, Friedrich Schiller University, Jena, Thuringia, Germany.

The 'friendship' of trees and fungi forming the close mutualistic symbiosis at tree roots does not only show its merit *via* the exchange of nutrients. Previous studies strongly suggested an initial mediation of tolerance towards several environmental stressors for the tree to ensure the benefit of the continuous carbohydrate flow from tree to fungus. These stressors include elevated metal concentrations in soil. This study presents the first holistic, comparative gene expression analysis within the established ectomycorrhiza challenged by elevated levels of various metals. Aim is to unravel symbiosis functioning considering both symbiotic partners by RNA-seq analyses. Test system will be an axenic co-culture of the basidiomycete *Tricholoma vaccinum* and the coniferous tree *Picea abies* (spruce). To elucidate relevant metal stress, the tolerance range of both mycorrhizal partners towards elevated levels of various metals were analyzed *in vitro* based on fungal radial growth and mycelial characteristics as well as tree seedling development. Additionally, a stress treatment with a redox cycling compound will supplement metal treatments to understand the impacts of radicals generated by the metal ion. Subsequent studies will provide the opportunity to identify and characterize metal stress associated genes and to establish stress markers for single metal species.

554. Yeast as tool to identify infection relevant genes in the phytopathogenic fungi. Susanna A. Braus-Stromeyer, Rebekka Harting, Tri-Thuc Bui, Van-Tuan Tran, Gerhard H. Braus. Georg August University, Goettingen, Germany.

Introduction: Fungal plant pathogens penetrate host cell walls as initial step for infection. Attachment of fungal pathogens to their hosts is mediated by adhesive proteins and is crucial during the host-parasite interaction. Hyphae of the ascomycete *Verticillium* attach directly to plant roots and enter the plants with the help of hyphopodia. The control mechanisms and the adhesive proteins involved in the initial infection process are not yet known. **Objectives:** We aimed to identify infection-relevant genes in the plant pathogen *Verticillium.* Genes involved in adhesion to the host surface were addressed in this study. **Methods:** We used a non-adherent mutant strain of the model yeast *S. cerevisae* to isolate genes required for *Verticillium longisporum* or *V. dahliae* adhesion and pathogenicity. Candiate genes were phenotypically analyzed by using genetics, cell biology, transcriptomics, secretome proteomics and plant pathogenicity assays. **Results**: Control elements for early plant infection were identified in a genetic screen and resulted in the discovery of six transcription regulatory genes of *Verticillium* (Vta1-Vta6) [1, 2]. All candidate genes were shown to reprogram non-adherent budding yeasts for adhesion. Vta2 is conserved in filamentous fungi, is required for fungal growth and is mandatory for accurate timing and suppression of resting structures (microsclerotia). *Verticillium* impaired in Vta2 is unable to colonize plants and induce disease symptoms. Vta2 controls expression of 270 transcripts and is a major regulator of fungal pathogenesis and controls H_2O_2 detoxification. Vta2 represents an interesting target to control growth and development of these vascular pathogens. **Outlook:** Additional candidates of the screen are the focus of our current research and we will include these data in our poster.

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[2)] Tran VT, Braus-Stromeyer SA, Timpner C, Braus GH (2013). Appl Microbiol Biotechnol: 4467–4483.

555. Transcriptional profiling of genes encoding secreted hydrolytic enzymes of *Zymoseptoria tritici* during in planta infection. Javier Palma-Guerrero, Stefano Torriani, Daniel Croll, Parvathy Krishnan, Bruce A. McDonald. Plant Pathology Group, Institute of Integrative Biology, ETH, Zurich, Switzerland.

Zymoseptoria tritici (previously called *Mycosphaerella graminicola*) is an ascomycete fungus that causes Septoria Tritici Blotch (STB), an important foliar disease on wheat. *Z. tritici* is an important pathogen in Europe where it is usually managed with fungicides. *Z. tritici* populations contain extremely high levels of genetic variability, enabling rapid adaptation to fungicides and resistant cultivars. *Z. tritici* infects plants through stomata rather than by direct penetration. After infection it exhibits a long asymptomatic incubation period of up to 2 weeks. The fungus evades host defenses during this phase, and is thought to derive its nutrition from the apoplast around living cells. The long incubation period is followed by a rapid switch to necrotrophy prior to formation of fruiting bodies 12–20 days after penetration. The factors associated with the switch from biotrophy to necrotrophy remain unknown. The *Z. tritici* reference genome contains a greatly reduced number of cell wall degrading enzymes (CWDEs) compared to other sequenced fungal genomes. It was proposed that nutrition during the asymptomatic phase is based on degradation of proteins in the apoplastic fluid rather than carbohydrates, which could be an evolutionary response to evade detection by plant defenses. Using RNA-seq data generated across the entire pathogen life cycle for the highly virulent Swiss strain ST99CH3D7 we analyzed the expression profile of genes encoding secreted hydrolytic enzymes including: CWDEs, Proteases, Lipases, Alpha amylases and Peroxidases. The results of our analyses support the previous theories about the importance of proteases during the asymptomatic phase, but also provide new insights into the role of different hydrolytic enzymes at different stages of the infection process. We identified new candidate genes that may play an important role in infection by the fungus.

556. Hydroxy Fatty Acids Sensing and Surface Perception by *Ustilago maydis*. Pierre Grognet, Regine Kahmann. Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

For *Ustilago maydis*, filamentous growth and appressoria formation are the key stages for infection. In nature, the switch from yeast to hyphae takes place on the plant surface in response to sensing plant signals. It is known that two different stimuli are sensed: hydrophobicity and hydroxy-fatty acids (HFAs). While two membrane proteins involved in hydrophobicity sensing are known (Msb2 and Sho1), nothing is known yet about the receptors involved in HFAs sensing. HFAs trigger filamentation of *U. maydis* and are required for

efficient appressoria formation on a hydrophobic surface. HFAs belong to a broader family of compounds called oxylipins. These molecules are present in all major groups of eukaryotes and play crucial roles in communication either between individuals or as internal communication signal. All known receptors for oxylipins are G protein-coupled receptors (GPCR). Mining the genome of *U. maydis* allowed us to identify 63 putative 7 trans-membrane domains proteins and spot candidate oxylipins receptors. Here we discuss the role of these candidate receptors in HFA sensing and provide insight into the downstream signaling pathways triggered by HFAs.

557. Regulation of secreted virulence factors by the Unfolded Protein Response. Martin Hampel[1], Florian Finkernagel[2], Gunther Doehlemann[3], Kai Heimel[1]. 1) Georg-August-University Goettingen, Institute for Microbiology and Genetics, Department of Molecular Microbiology and Genetics, 37077 Goettingen, Germany; 2) Philipps-University Marburg, Institute of Molecular Biology and Tumor Research, Emil-Mannkopff-Strasse 2, 35032 Marburg, Germany; 3) Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strabe 10, 35043 Marburg, Germany.

The basidiomycete *Ustilago maydis* is the causative agent of smut disease on its host plant *Zea mays*. The infection process requires the morphogenetic transition from budding to filamentous growth. Proliferation *in planta* is dependent on the interaction between the developmental regulator Clp1 and the Hac1-like transcription factor Cib1. Cib1 represents the master regulator of the unfolded protein response (UPR), a conserved eukaryotic signaling pathway to protect cells from endoplasmic reticulum stress. During biotrophic interaction, development-specific activation of the UPR coincides with elevated expression of large gene sets encoding secreted effector proteins that function to establish a compatible biotrophic interaction and support fungal growth *in planta*. The UPR facilitates efficient secretion of effector proteins by re-structuring of the secretory pathway to boost the cellular capacity for protein secretion. Consistently, UPR mutant strains are fully avirulent and provoke increased plant defense responses. However, to our suprise we also observed UPR-dependent transcriptional regulation of various effector proteins, including Pit1 and Pit2, both of which are crucial for virulence. Targeted deletion of predicted UPR elements (UPREs) in the divergent *pit1/pit2* promoter resulted in loss of UPR-dependent *pit1/pit2* expression, indicating a direct regulation of *pit1/pit2* by Cib1. Thus, increased plant defense responses observed upon infection with UPR mutant strains might result from altered ER stress response as well as a non-functional transcriptional regulation of genes encoding effector proteins. Our results expand the role of the UPR as a signal hub in fungal virulence and illustrate how biotrophic fungi can coordinate cellular physiology, development and regulation of secreted virulence factors.

558. Structure-function analysis of Cmu1, a secreted chorismate mutase in *Ustilago maydis*. Xiaowei Han[1], Jan Schuhmacher[2], Anupama Ghosh[1,3], Armin Djamei[1,4], Regine Kahmann[1], Gert Bange[2]. 1) MPI for Terrestrial Microbiology, Marburg, Germany; 2) LOEWE Center for Synthetic Microbiology (Synmikro), Marburg, Germany; 3) Division of Plant Biology, Bose Institute, West Bengal, India; 4) Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria.

The smut fungus *Ustilago maydis* codes for more than 300 secreted effectors which are mostly novel and contribute to the establishment of its biotrophic interaction with its host maize. These effectors exert their function either in the apoplast or translocate into the plant cells. The secreted chorismate mutase Cmu1 of *U. maydis* is one such translocated effector. Cmu1 is involved in suppressing host immunity by lowering salicylic acid levels. How Cmu1 is delivered into the plant cell is currently unknown. To obtain insight into this we have mapped a domain in Cmu1 that is not needed for its catalytic activity, but contributing to function in *U. maydis*. We have determined the structure of Cmu1 by X-ray crystallography using *E. coli* expressed protein and have initiated a detailed structure-based functional analysis. The structure revealed an acidic, surface-exposed patch which might be involved in the uptake of Cmu1. Two conserved cysteine residues, which are unique in secreted chorismate mutases of smut fungi, form a disulfide bridge, which stabilizes the overall fold of the protein. In addition, the structure provides new insights into the allosteric regulation of Cmu1.

559. The Unfolded Protein Response functions as a signal hub for biotrophic development of *Ustilago maydis*. Kai Heimel[1], Martin Hampel[1], Johannes Freitag[2], Julia Ast[2], Florian Finkernagel[3], Gunther Doehlemann[4], Michael Bölker[2], Jörg Kämper[5]. 1) Department of Molecular Microbiology and Genetics, Georg-August-University, Goettingen, Germany; 2) Department of Genetics, Philipps-University, Marburg, Germany; 3) Institute of Molecular Biology and Tumor Research, Marburg, Germany; 4) Department of Terrestrial Microbiology, University of Cologne, Germany; 5) Department of Genetics, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

The infection process of the biotrophic basidiomycete *Ustilago maydis* requires the coordination of various signalling pathways. To cause disease the infection related morphogenetic program, sexual development and virulence factor delivery have to be aligned with fungal physiology . We identified the unfolded protein response (UPR), a pathway to counteract secretion stress, as a regulatory hub connecting these processes. During pathogenic development the UPR is specifically activated at the biotrophic stage. Premature UPR-activation interferes with morphogenetic switching and pathogenic development. By contrast, biotrophic development *in planta* requires an active UPR pathway and is governed by the physical interaction between the Hac1-like UPR regulator in *U. maydis* (Cib1), and the central regulator of fungal development *in planta*, Clp1. As a result of this interaction (i) Clp1 is stabilized, triggering fungal proliferation *in planta* and (ii) UPR signalling is modified to confer hyper-resistance towards ER stress, allowing for extended UPR activation during biotrophic development. UPR-mutants elicit increased plant defence response and analysis of UPR-dependent gene expression revealed various secreted virulence factors to be regulated by the UPR. Hence, the UPR is used to synchronize developmental processes and secretion of effector molecules to rapidly establish a compatible plant/fungus interaction.

560. Intracellular and potential extracellular roles of the *Ustilago maydis* Acyl-CoA-binding protein Acb1. Joachim Jungmann[1], Till Ringel[1], Stefanie Reiβmann[1], Christophe Anjard[2], Christine Trippel[1], Friederike Hartwig[1], Regine Kahmann[1]. 1) Organismic Interactions, MPI for Terrestrial Microbiology, Marburg, Hessen, Germany; 2) Centre de Génétique et de Physiologie Moléculaire et Cellulaire, 16 Rue Raphel Dubois, F-69622 Villeurbanne, France.

Conventionally secreted fungal effectors 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 established that proteins without a signal peptide can also be targeted to the

outside of the cell in an ER/ Golgi independent manner. Many of these unconventionally secreted proteins have been shown to be 'moonlighting' proteins with an extracellular function distinct from their intracellular function. One candidate for an unconventionally secreted protein in *U. maydis* is the UmAcb1 protein, previously detected in the apoplastic fluid of infected maize. UmAcb1 is an ortholog to the *Dictyostelium discoideum* acyl-CoA-binding protein AcbA, which is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) that triggers terminal spore differentiation upon interaction with a membrane receptor [1].

In our work we attempt to prove the secretion of *U. maydis* Acb1 and its processing into an SDF-2 like peptide, as well as demonstrate a function for the peptide. We could show that *U. maydis* hyphal culture supernatants, as well as apoplastic fluid extracted from *U. maydis* infected plants, trigger spore formation in a *D. discoideum* based bioassay indicating the secretion of an SDF-2-like peptide by *U. maydis*. Deletion of the *acb1* gene in *U. maydis* attenuated growth and led to a mating defect. Both phenotypes could be rescued by suppressor mutations, presumably bypassing the intracellular function. We are now exploiting these suppressors to analyze the putative extracellular function of UmAcb1.

Duran et al., 2010. Unconventional secretion of Acb1 is mediated by autophagosomes. J Cell Biol. 2010 188(4):527-36.

561. Hxt1, a monosaccharide transporter and sensor required for virulence of the maize pathogen *Ustilago maydis*. David Schuler[1], Ramon Wahl[1], Kathrin Wippel[2], Norbert Sauer[2], Joerg Kaemper[1]. 1) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Molecular Plant Physiology, Friedrich Alexander University, Erlangen, Germany.

Ustilago maydis, a ubiquitous pest of corn, is highly adapted to its host to parasitize on its organic carbon sources. We have identified a hexose transporter, Hxt1, as important for fungal development both during the saprophytic and the pathogenic stage of the fungus. Hxt1 is a high affinity transporter for glucose, fructose and mannose; *Δhxt1* strains show significantly reduced growth on these substrates, setting Hxt1 as the main hexose transporter during saprophytic growth. After plant infection, *Δhxt1* strains show decreased symptom development. However, expression of a Hxt1 protein with a mutation (R164K) that leads to constitutively active signaling in the yeast glucose sensors Snf3p and Rgt2p results in completely apathogenic strains. Development of strains expressing the Hxt1(R164K) allele are stalled immediately after plant penetration, implying a second function of Hxt1 as sensor. As glucose sensors are only known for yeasts, "transceptors" as Hxt1 may constitute a general mechanism for sensing of glucose in fungi. In *U. maydis*, Hxt1 links a nutrient-dependent environmental signal to the developmental program during pathogenic development.

562. Characterization of the *Ustilago maydis* pathogenicity factor Scp2 - a candidate for unconventional secretion. Sina Krombach, Stefanie Reissmann, Florian Bochen, Regine Kahmann. MPI for Terrestrial Microbiology, Marburg, Germany.

The fungal pathogen *U. maydis* relies on the secretion of effector proteins to establish and maintain a biotrophic interaction with its host plant *Zea mays*. In recent years it has been discovered that proteins lacking a classical signal peptide can also be targeted to the extracellular space by mechanisms that do not depend on the endoplasmic reticulum and the Golgi complex. We want to identify such so called 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, previously detected in the apoplastic fluid (APF) of infected maize leaves. One of the identified candidate proteins is Scp2. Scp2 harbors a peroxisomal targeting signal and shares 36 % amino acid identity with the human sterol carrier protein 2 (SCP2). The *scp2* gene is up-regulated during plant colonization and deletion of *scp2* results in a virulence defect that appears to result from a reduced efficiency of plant penetration. This penetration defect cannot be attributed to a defect in peroxisomal β-oxidation. Based on our finding that peroxisomal targeting of Scp2 is crucial for its virulence-related function we speculate that Scp2 could either function exclusively in peroxisomes or exhibit a dual function in peroxisomes and in the extracellular space.

563. The role of Stp1, a secreted effector of *Ustilago maydis* essential for host colonization. Liang Liang[1], Kerstin Schipper[1,2], Libera Lo Presti[1], Regine Kahmann[1]. 1) MPI for Terrestrial Microbiology, Dept. Organismic Interactions, Marburg, Hessen, Germany; 2) Heinrich Heine University Düsseldorf, Dept. Microbiology, Düsseldorf, Germany.

Secreted protein effectors play crucial roles during successful establishment of pathogens in their host plants. In the corn smut fungus *Ustilago maydis*, one of these essential effectors is *stp1*. *stp1* mutants are non-pathogenic and arrest shortly after penetration. Deletion analysis revealed that the N- and C-terminal domains of Stp1 are essential for function while the large central region is dispensable. In addition, co-expression of secreted separated N- and C-terminal domains of Stp1 could restore pathogenicity of a *Δstp1* strain. To elucidate the function of Stp1, we have identified 20 putative maize interactors (Sip proteins) of Stp1 by yeast two-hybrid screening. The activity of Sip3, a secreted maize cysteine protease, could be inhibited by both $Stp1_{\Delta 136\text{-}432}$ (lacking the central domain) as well as the C-terminal domain of Stp1, $Stp1_{433\text{-}515}$. However, the biological relevance of this inhibition is still elusive as we could not complement the *stp1* mutant with Pit2, another effector targeting the same cysteine protease (Mueller *et al.*, 2013). Additionally, the activity of Sip19, a serine/threonine-protein kinase isolated as putative cytoplasmic interactor of Stp1 could be inhibited by both $Stp1_{\Delta 136\text{-}432}$ and $Stp1_{433\text{-}515}$. When transiently expressed in maize, Stp1 localized to the nucleus. However, a Stp1 protein fused to a nuclear export signal could complement the *stp1* mutant, suggesting that the nuclear localization is not essential for the function of Stp1. A newly established uptake assay based on transgenic maize expressing cytoplasmic BirA biotin ligase suggests that Stp1 is an apoplastic effector. These latter experiments raise serious doubts on the presumed cytosolic function of Stp1. We are currently attempting to localize Stp1 via immuno-EM and discuss possible reasons why yeast two-hybrid screening may have failed to detect genuine interactors of Stp1.

564. The secreted repetitive protein Rsp3 of *Ustilago maydis* is required for full virulence. Lay-Sun Ma[1], Christine Trippel[1], Lei Wang[2], Artemio Mendoza-Mendoza[3], Steffen Ullmann[4], Marino Moretti[1], Stefan Wawra[5], Stefanie Reissmann[1], Karin Münch[1], Regine Kahmann[1]. 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Max Planck Institute for Heart and Lung Research, Department of Pharmacology, D-61231 Bad Nauheim, Germany; 3) Protection Research Centre PO Box 64, Lincoln University. Lincoln 7647, New Zealand; 4) Heinrich-Heine-Universität Düsseldorf, Molekulare Mykologie, Universitätsstrabe 1, D-40225 Düsseldorf, Germany; 5) University of Cologne, Botanical Institute, Zülpicher Str. 47a, 50674 Cologne, Germany.

The biotrophic fungus *Ustilago maydis* causes smut disease in maize. Hallmarks of the disease are the formation of tumor and anthocyanin induction. Here, we show that the repetitive secreted protein Rsp3 is required for virulence and anthocyanin accumulation. Rsp3 consists of several repetitive as well as a Cys-rich domain and is highly expressed during the biotrophic stage. Interestingly, *rsp3* alleles obtained from field isolates of *U. maydis* differ in size ranging from 1.8 to 2.5kb. These size differences are caused by reduced or expanded numbers of certain repeats. The shortest *rsp3* allele from a strain collected in Toluca valley in Mexico into the *U.* maydis *rsp3* mutant, could fully restore virulence, illustrating full functionality. Rsp3 can be easily be detected in culture supernatants when expressed from a constitutive promoter. The protein showed highly anomalous migration behavior on SDS-PAGE that did not depend on the presence of the Cys-rich domain. Furthermore, deletion of the Cys-rich domain of Rsp3 prevented tumor induction but anthocyanin induction was still observed. This suggests that Rsp3 could be a dual function effector.

565. Sensing the plant surface: investigating the activation mechanism of Msb2 in *Ustilago maydis*. Marino Moretti, Daniel Lanver, Alexander Carsten, Irina L. Schmidt, Regine Kahmann. MPI for Terrestrial Microbiology, Marburg, Germany.

The transmembrane mucin protein Msb2 acts upstream of the Kpp2/Kpp6 pathogenicity-related MAP kinase cascade in *U. maydis*. Msb2 regulates the differentiation of appressoria in response to the hydrophobicity of the plant surface and deletion mutants are strongly attenuated in virulence. Genome-wide transcriptional profiling revealed that secreted proteins including plant cell wall degrading enzymes and effectors are regulated by Msb2. In *Saccharomyces cerevisiae* and *Candida albicans* Msb2 is processed into a secreted and a cell-associated form, and cleavage is required for the signaling function. In *U. maydis*, Msb2 is similarly processed but our results obtained so far do not support that processing leads to an activation of Msb2. In appressoria, where Msb2 is presumed to be active, the protein is mainly detected as full-length, while in axenic culture the protein is detected predominantly in its processed form. This may suggest that uncleaved protein is the active form and processing may be related to protein turnover. In yeast and *C. albicans*, Msb2 is cleaved by an aspartic protease. To gain insights into the activation mechanism of Msb2 in *U. maydis*, we have mutagenized the region containing the presumed cleavage site. In addition, we have generated single and multiple aspartyl protease deletion mutants in *U. maydis* to test for their role in Msb2 processing. Finally, a mass-spectrometry approach is on-going to define the cleavage site in Msb2. All results will be presented and discussed.

566. Necroptrophic effector epistasis in tan spot of wheat. L.M. Ciuffetti, V.M. Manning, I. Pandelova. Dept Botany & Plant Pathology, Oregon State Univ, Corvallis, OR.

Pyrenophora tritici-repentis, the causal agent of tan spot disease of wheat, has been identified in major wheat growing areas worldwide and tan spot is considered a disease of economic importance. Tan Spot has emerged as an experimental model for the study of diseases that conform to an inverse gene-for-gene relationship where pathogenicity/virulence has been causally associated with the production of multiple host-selective toxins (HSTs). The importance of HSTs in disease development indicates that the loss of any of these pathogenicity factors would cause a reduction in virulence when the pathogen expresses multiple HSTs to which the host is sensitive. However, following deletion of the gene encoding the HST Ptr ToxA (ToxA) or the heterologous expression of *ToxA* in a race that previously did not produce this toxin, we demonstrate that ToxA symptom development is epistatic to other HST-induced symptoms. Data from this study will be presented and indicate a complex interaction between host responses and at least some HSTs. To our knowledge, this is the first demonstration of necrotrophic effector epistasis.

567. Characterization of a *Pyrenophora teres* f. *maculata* mapping population uncovers the complexity of virulence in the spot form net blotch of barley interaction. T.L. Friesen[1,2], S.A. Carlsen[2], J.K. Richards[2], A. Neupane[2], R.S. Brueggeman[2]. 1) Cereal Crops Res Unit, USDA-ARS, Fargo, ND; 2) Department of Plant Pathology, North Dakota State Univ., Fargo, ND USA.

Pyrenophora teres f. *maculata* is a major pathogen of barley worldwide, however, little is known about the virulence underlying this disease. Based on its necrotrophic lifestyle, the pathogen likely produces several necrotrophic effectors (NEs) that elicit NE triggered susceptibility (NETS) in the host. To gain insight into the virulence of *P. teres* f. *maculata*, a mapping population was developed using a cross between *P. teres* f. *maculata* isolates FGOB10ptm (North Dakota) and SG1 (Australia) to derive 105 progeny. The population was phenotyped using nine diverse barley lines including common SFNB differential lines and lines from the world barley core collection put together by the Triticeae Coordinated Agriculture Project (T-CAP). Lines were selected that demonstrated differential reaction to the selected parental isolates. The North Dakota isolate had significantly higher virulence on seven of the barley lines while the Australian isolate had higher virulence on two of the lines. Genotyping was done using a two-enzyme restriction associated DNA (RAD) GBS approach developed for the Ion Torrent PGM. A SNP calling pipeline identified a total of 983 quality SNP markers. These SNP markers were used to develop the first genetic map of *P. teres* f. *maculata*. Co-segregating markers were eliminated leaving 488 informative markers. Markers were distributed across 17 linkage groups generating a total map size of 1780 cM. Using phenotypic and genotypic data, QTL analysis identified more than 20 genomic regions on seven of the linkage groups, associated with *P. teres* f. *maculata* virulence. QTL associated with individual barley lines ranged from three to seven with each line used showing a different QTL pattern. The variation in virulence QTLs between barley lines and the high number of genomic regions associated with virulence indicates a high level of complexity in both pathogen virulence and host resistance or susceptibility.

568. Using the phenotypic information in the Pathogen-Host Interactions database (PHI-base) to explore pathogen genomes, transcriptomes and proteomes. Martin Urban, Alistair Irvine, Alayne Cuzick, Kim Hammond-Kosack. Dept of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts, United Kingdom.

The PHI-base database (www.phi-base.org) stores expertly curated molecular and biological information on numerous virulence and effectors genes for which the effect on pathogen-host interactions has been tested experimentally. Fungal, protist and bacterial pathogens and nematodes which infect plant, animal, fish, insect and/or fungal hosts are included. Information is also given on the target sites of some anti-infective chemistries [1]. PHI-base phenotypic information is provided to Ensembl and mapped to pathogen genomes (www.ensemblgenomes.org).

PHI-base now provides information > 2,800 pathogen genes described in 4000 pathogen-host interactions (80% plant hosts), involving 160 pathogenic species. Each curated entry in PHI-base is checked by individual species experts and is supported by strong experimental evidence (e.g. gene disruption/deletion, complementation experiments) and literature references.

Several case studies will be presented to show how the phenotypic data in PHI-base can be used to enrich *in silico* predictive, comparative genomic and / or network analyses as well as chemogenomic, transcriptomic, proteomic and protein-protein interaction analyses. The PHI-base entries can also be used to fast track the interpretation of results from forward and reverse genetic experimentation.

In 2015 our aim is to include the curation of the extra- and intracellularly localised host plant targets of pathogen effectors. We also aim to provide an online community curation tool for phenotypes, genetic and molecular information from your paper directly into PHI-base.

PHI-base receives BBSRC support as a National Capability and from PhytoPath BBR project done in collaboration with the EBI Cambridge, UK. [1] Urban *et al.* (2015) Nucleic Acids Research, Database issue (Jan).

569. An extracellular DNase from the phytopathogen *Cochliobolus heterostrophus* is a virulence factor as found for bacterial pathogens of animals. Weiwei Wang[1], Gilberto Curlango-Rivera[3], Zhongguo Xiong[2], Hans VanEtten[2], B. Gillian Turgeon[1], Martha Hawes[3]. 1) Plant Pathology & Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY; 2) School of Plant Sciences, Univ. of Arizona, Tucson, AZ 85721; 3) Dept of Soil Water,& Environmental Science, Univ. of Arizona, Tucson, AZ 85721.

During the past decade, new insight into fundamental mechanisms of mammalian immunity emerged when neutrophils were found to function by a process distinct from those believed to predominate in defense (Science 303:1532). Histone-linked extracellular DNA (exDNA) is an integral component of neutrophil extracellular traps (NETs), and when this exDNA is degraded by addition of DNase I, the capacity to immobilize and kill invading pathogens is lost. As a counter defense, bacterial pathogens have been shown to produce extracellular DNases (exDNases): mutation of Group A *Streptococcus* exDNases results in reduced trapping and systemic spread in animal hosts (Curr Biol 16: 396). Extracellular trapping has now been documented for diverse animal tissues, and shown to play a role in disease response to bacteria, fungi, viruses, and protozoan parasites. We have investigated whether or not a similar mechanism functions in higher plant cell defense. Initially, we demonstrated that plant cells carry out an extracellular trapping process that appears to function in a manner parallel to that of mammalian cells. Histone-linked exDNA can be found as an integral component of a matrix secreted from root caps along with specialized root 'border cells' that trap soilborne bacteria, fungi, and heavy metals. Furthermore, exDNA is required for root tip resistance to fungal infection (Plant Sci 180:741). Here, we report, for the first time to our knowledge, that targeted deletion of a gene encoding a putative exDNase in a fungal pathogen is correlated with reduced virulence of the mutant on its plant host. Thus, a fungal exDNase is a virulence determinant. Overall these data provide support for a common defense/counter defense mechanism used by animals, plants and their pathogens.

570. Fast(er) forward genetics screening to identify virulence-associated genes in *Zymoseptoria tritici*. Juliet Hooper, Robert King, Jason Rudd. Rothamsted Research, Harpenden, Herts, AL5 2JQ United Kingdom.

Zymoseptoria tritici (aka, *Septoria tritici / Mycosphaerella graminicola*) is a Dothideomycete fungal pathogen of cultivated wheat, and the causal agent of Septoria tritici blotch (STB) disease. *Z. tritici* is considered to be dimorphic, as it exhibits environmentally regulated morphogenetic switching between budding “yeast-like” and hyphal growth. The switch to hyphal growth is essential for virulence allowing the fungus to penetrate plant stomata and colonise the intercellular leaf spaces. Despite its agricultural importance, to date <45 genes have been identified which contribute to virulence of *Z. tritici* (see www.PHI-base.org). The availability of a finished genome sequence and efficient Agrobacterium-mediated transformation protocols for the reference isolate IPO323, now enable T-DNA mutagenesis screens to be performed with the aim of identifying novel fungal virulence genes. We have generated a library of ~1,200 T-DNA insertion mutants of *Z. tritici* isolate IPO323. Initial screens for virulence defects on ~400 mutants using attached wheat leaf infection assays identified several with reduced virulence (1). However the extended time taken for completion of plant infection rendered the screening process slow. For this reason the remaining mutants were pre-screened for defects in yeast to hyphal growth switching. Five mutants affected in this process were identified and all five exhibited reduced virulence when subsequently tested on wheat leaves. T-DNA insertion sites within predicted ORF’s, each encoding enzymes, were detected via TAIL-PCR, and this highlighted that at least three different biological processes contribute to the infection related growth transition. We are currently further characterising the genes affected and exploring the use of next generation whole genome re-sequencing to further speed up forward genetic analyses for *Z. tritici* (see meeting poster Urban et al.).

(1) Aberrant protein *N*-glycosylation impacts upon infection-related growth transitions of the haploid plant-pathogenic fungus *Mycosphaerella graminicola.* (2011) Molecular Microbiology **81** (2), p 415–433.

571. Identification of pathogenicity and virulence genes by random T-DNA insertional mutagenesis in *Cochliobolus sativus*. Shaobin Zhong[1], Abdollah Ahmadpour[1,2], Rui Wang[1], Yueqiang Leng[1]. 1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA; 2) Department of Plant Protection, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Cochliobolus sativus (*Bipolaris sorokiniana*) is the causal agent of three important diseases (spot blotch, common root rot and black point) in barley and wheat. However, molecular mechanisms of host-pathogen interactions in this pathosystem are poorly understood. To identify genes related to pathogenicity and virulence in *C. sativus*, we generated a T-DNA insertional mutant library by *Agrobacterium tumefaciens*–mediated transformation (ATMT). A binary plasmid vector (pBIN19) containing the hygromycin B phosphotransferase (*hph*) under the control of the *cpc-1* promoter from *Neurospora crassa* was introduced into *A. tumefaciens* strain AGL-1. The bacterial cells with pBIN19 were pre-treated with acetosyringone and then co-cultivated with the conidia of the *C. sativus* isolate ND4008, which exhibits high virulence on the three barley differential lines Bowman, ND5883 and NDB112. A total of 6,367 transformants were generated from ND4008. Analysis of a subset of transformants with PCR and Southern hybridization showed that most of them contained a single copy of the T-DNA insertion. Among 1000 transformants tested on a barley line susceptible to the wild type isolate ND4008, 32 were significantly reduced in virulence. One of these mutants with morphological characters similar to those of wild type is being characterized for the gene sequence disrupted by the T-DNA insertion. The results will be presented in the meeting.

572. Exploring the concept of non-susceptibility in the *Parastagonospora nodorum* – wheat interaction. Peter Solomon, Lauren Du Fall, Adam Taranto. Research School of Biology, Australian National University, Canberra, ACT, Australia.

More often than not, we judge the outcome of plant-pathogen interaction as either being resistant or susceptible. Resistance is thought to typically occur at one of two levels, PAMP- or effector-triggered immunity (PTI and ETI). In PTI, plants detect signals common to pathogens such as flagellin or chitin. This in turn triggers the induction of a quantitative defence response inhibiting the growth of the pathogen. In the event that the pathogen is able to circumvent PTI, pathogen effector proteins or molecules can be detected though specific gene-for-gene mechanisms leading to ETI resulting in a stronger defence response. As such, a constant evolutionary battle exists between the host resistance proteins and pathogen effectors to mediate recognition/non-recognition.

However, there is another outcome of plant-pathogen interactions that should also be considered; non-susceptibility. In the case of *Parastagonospora nodorum*, we now know that the pathogen causes disease by secreting effector proteins that are recognised by the host (wheat) resulting in cell death and a conducive environment for the necrotrophic pathogen to thrive and cause disease. But what happens in the absence of these host susceptibility genes? In the example of the *P. nodorum*-wheat interaction, we know that non-susceptible leaves are asymptomatic, but the pathogen remains viable inside the leaf for several weeks. Such data suggests that the pathogen is able to escape the PTI defence response.

In this poster, I will present recent work where we have undertaken a comprehensive RNA-seq analysis of a *Parastagonospora nodorum* infection on a susceptible and non-susceptible host. Multiple early time points have been analysed and the expression of both pathogen and host genes assessed. These data have provided a unique insight into not only pathogenicity mechanisms, but also the basis of non-susceptibility.

573. Genome and transcriptome analyses of the fungal forest pathogen *Armillaria ostoyae*. Martin Münsterkötter[2], Mathias Walter[3], Ulrich Güldener[2,3], György Sipos[2,1]. 1) University of West-Hungary, Sopron, Hungary; 2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany; 3) Department of Genome oriented Bioinformatics, Technische Universität München, Wissenschaftszentrum Weihenstephan, Maximus-von-Imhof Forum 3, 85354 Freising, Germany.

Armillaria (*Basidiomycota, Agaricomycetes*) species are primary decay drivers in forest ecosystem processes. Their diverse ecological habits, besides beneficial saprotrophic activities, include symbiotic mycorhizzal and invasive necrotrophic interactions with their hosts. The aggressive pathogenic *Armillaria* species represents one of the most prominent killers, decayers of broad-leaved and coniferous trees all over the world.

The genome of the devastating forest pathogen *Armillaria ostoyae* was *de novo* sequenced employing PacBio 'long-read' and Illumina 'short-read' paired-end technology. The final assembly yielded 280 contigs, with the total length of 61.48 Mb, of which 36 supercontigs covered nearly 90% of the genome.

To assess adaptive genomic and transcriptomic traits to its environment we conducted comparative genomics, developed an *in vitro* assay to test virulence and analysed the differential genome expression profiles in virulent and non-virulent isolates under invasive versus saprotrophic conditions in the *Armillaria*-Norway spruce pathosystem.

Comparative genomics using other selected *Basidiomycota* species, representing various functional ecotypes, revealed that *A. ostoyae* has a distinctive potential in degrading pectins and aromatic compounds. The *in vitro* transcriptome analysis highlighted the differential expression of hydrophobins. Furthermore, all genes encoding phytotoxic cerato-platanins and a significant number of secreted enzymes involved in aromatic intradiol-ring cleavage, were higher expressed in the virulent isolate when invading the cambium of fresh stems. Our findings confirm the adaptive invasive character of *Armillaria ostoyae* as a white rot fungus with a facultative necrotrophic lifestyle.

Population and Evolutionary Genetics

574. A regular unicellular stage facilitates adaptation: the emergence of azole resistance in *Aspergillus fumigatus* . Jianhua Zhang[1], Alfons Debets[1], Paul Verweij[2], Willem Melchers[2], Bas Zwaan[1], Sijmen Schoustra[1]. 1) Laboratory of Genetics, Wageningen University, Wageningen, Netherlands; 2) Department of Medical Microbiology, Radboud University Medical Centre, 6500 HB Nijmegen, The Netherlands.

Understanding the occurrence and spread of azole resistance in *Aspergillus fumigatus* is crucial for public health. It has been hypothesized that a unicellular stage via asexual sporulation is essential for phenotypic expression of azole resistance mutations in *A. fumigatus* facilitating subsequent spread through natural selection. We assessed the advantage of unicellular stage via asexual sporulation by growing the fungus under pressure of one of five different azole fungicides and monitoring the rate of adaptation between scenarios of, (i) exclusive mycelium growth (multicellular stage) without asexual sporulation, and, (ii) growth allowing asexual sporulation (unicellular stage). Our results unequivocally show that unicellular stage via asexual sporulation enhances adaptation over a fixed period of evolutionary time. This can be explained by the combination of more effective selection because of the transition from a multicellular to a unicellular stage, and by increased mutation supply due to the sporulation process, which involves numerous mitotic divisions. Our insights are essential to unravel the fungal adaptation strategies are highly relevant for resistance development in the natural environment, but also for patients with chronic aspergillus diseases. Uncovering the pathways to adaptation will help to improve our current management strategies both in agricultural and medical fields.

Key words: *Aspergillus fumigatus*; azole resistance; unicellular and multicellular; asexual sporulation; experimental evolution; MIC value; mycelial growth rate.

575. Identification of novel genes regulating sexual reproduction in *Aspergillus* species. N Salih, P. Dyer. University of Nottingham, Nottingham, United Kingdom, NG7 2RD.

Having an understanding of the reproductive mode of an organism is of great importance for gaining insights into the potential for the evolution of a given species, whilst the sexual cycle also provides a valuable tool for strain improvement of species used in industrial applications. About one third of *Aspergillus* species are reported to be capable of sexual reproduction, but the majority of species are only known to reproduce asexually. However, sexual cycles have recently been discovered in a number of *Aspergillus* species that were previously thought to be strictly asexual. This has provoked increased research interest both in the possible genetic basis of asexuality and the molecular genetic control of sexual development. Studies are therefore in progress.to identify and characterise novel genes involved in sexual reproduction of *Aspergillus* species, to provide insights into sexual development and identify candidate genes possibly linked to asexuality. In previous work a set of genes had been identified that was differentially expressed in *MAT1-1* and *MAT1-2* mating-type strains of *A. oryzae*, most of which were of unknown function. Homologous gene were identified and then deleted from the homothallic (sexually self fertile) *A. nidulans* by targeted gene replacement in a Delta *nkuA* strain. Transformant strains were tested for sexual fertility to investigate the effect of gene deletion on sexuality. A range of effects was observed, from no obvious impact, to moderate loss or gain of fertility, to complete loss of sexuality. A gene complementation test, restoring the gene of interest, was then performed to confirm that observed major changes in sexuality were due to the deletion of target gene.

576. Succinate-dehydrogenase inhibitors (SDHIs) resistance evolution in cereal pathogens. Stefano Torriani, Regula Frey, Jürg Wullschleger, Carolina Buitrago, Stephane Bieri, Gabriel Scalliet, Helge Sierotzki. Syngenta, Stein, Switzerland.

Disease control is critical to cereal production in Europe; several commonly occurring diseases pose significant threat to yields and grain quality. In high disease pressure year, yield loss can be significant. Nowadays, cereal pathogens are mainly controlled by quinone outside inhibitors (QoIs), succinate-dehydrogenase inhibitors (SDHIs), demethylation inhibitors (DMIs) and multi-site fungicides, such as chlorotalonil. Resistances have been reported to the major fungicide classes. Therefore, understanding of evolutionary processes responsible for emergence and spread of resistance coupled to long term monitoring are essential to infer adequate anti-resistance management strategies. SDHIs block the TCA cycle at the level of succinate to fumarate oxidation, leading to an inhibition of respiration. SDHI resistance has been observed in 14 fungal pathogens to date and is caused by different mutations in the target genes encoding for the mitochondrial succinate dehydrogenase (SDH) enzyme. The SDH enzyme is composed by four subunits encoded by distinct genes (sdh-A to D). Mutations in sdh-B to D associate in different cereal pathogens to decreased SDHI sensitivity. Recently, *Zymoseptoria tritici* isolates carrying mutations T79N and W80S in sdh-C and N225T in sdh-B were reported. These mutations confer only limited resistance level. In *Pyrenophora teres* the first resistance allele H277Y in sdh-B was reported in 2012. In 2013 mutation associated to decreased sensitivity were found in all three SDH subunits (sdh-C G79R was the most frequent). In this study we describe the evolutionary forces shaping SDHI resistance in field and we will discuss the evolution of single target genes and how their combination into a functional enzyme might shape the response to fungicide.

577. Time for a background check: The importance of genetic background on the acquisition of drug resistance in *Candida albicans*. Aleeza Gerstein[1], Judith Berman[1,2]. 1) University of Minnesota, Minneapolis, MN; 2) Tel Aviv University, Israel.

Many fungal infections are successfully treated and cleared, yet breakthrough infection occur with appreciable and increasing frequency. The correlation between *Candida albicans* isolate MIC and patient outcome is not perfect - a considerable number of susceptible isolates do not respond to drug as expected. Fluconazole, the primary antifungal drug used to treat candidiasis, primarily inhibits growth rather than kills cells, thus population sizes can remain high, even in the face of considerable levels of drug. To examine the influence of genetic background on the acquisition of drug resistance we conducted an exerimental evolution study using 20 different clinical isolates. Replicates from each isolate were exposed to 1ug/mL fluconazole for ~100 generations. Although the MIC of most isolates was initially below this level, the majority of isolates were fairly tolerant to this low level of drug. Accordingly, after exposure, few large improvements

in fitness were identified, though the majority of strain replicates did exhibit small, but significant, growth improvements at low levels of fluconaozle. Interestingly, we identified a sharp disconnect between phenotypic variation and fitness improvements. Although changes in resistance were rare, phenotypic variability was pervasive after exposure to fluconaozle. Ploidy variation following drug exposure was frequently observed, particularly for isolates that were initially the least resistant. A second phase of the evolution experiment is currently underway to assess whether replicates that have been exposed to low levels of fluconazole are more likely to acquire resistance than ancestral strains when exposed to a higher level of fluconazole.

578. Minisatellites in IGS used for race differentiation in *Colletotrichum* of lentil and site of small RNA synthesis affecting pathogenicity. Jonathan Durkin[1], John Bissett[2], Rui Song[1], Hadi Pahlavani[3], Brent Mooney[1], Lone Buchwaldt[1]. 1) Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada; 2) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, Canada; 3) Department of Agronomy and Plant Breeding, Gorgan University of Agricultural Sciences, P.O. Box 386, Gorgan, Iran.

Colletotrichum truncatum (auct.) causes lentil anthracnose in Canada. A PCR-based DNA probe was developed to differentiate two races, Ct0 and Ct1, currently identified by inoculation of differential lines. PCR-based primers were used to amplify exon-intron spanning regions in *tef1α*, *rpb2*, *acla*, IGS and ITS regions. Sequence alignments were monomorphic in genic regions and ITS. IGS revealed two polymorphic minisatellites of 23 and 39 nucleotides (nt). A new primer, 39F/R, amplified length variants of the 39 nt repeat that were separated by gel electrophoresis. Race Ct1 having 7 or 9 repeats were consistently differentiated from race Ct0 having 2 to 12 repeats, but never 7 or 9. The 39F/R probe was employed to survey isolates from lentil seed and showed that race Ct0 currently constitute 95% likely because varieties lack Ct0 resistance, while some have resistance to race Ct1. The 39 nt minisatellite was located upstream of the 18S gene in *C. truncatum* and was identified in other fungal plant pathogens, *C. gloeosporioides*, *C. trifolii*, *Ascochyta lentis, Sclerotinia sclerotiorum* and *Botrytis cinerea.* All length variants formed single stranded stem-loop structures with homology to RNA polymerase termination factors pointing to involvement in regulation of ribosomal gene transcription. The other minisatellite had ten different repeats of a 23 nt sequence assembled into three length variants. Race Ct1 had 17 repeats, while Ct0 had either 14 or 19 repeats, except one isolate. RNA translation of each 23 nt repeat had uracil in position 1 and formed stable hairpin structures indicative of small RNA synthesis. Some fungal derived small RNA can silence genes either in the host or pathogen which in both cases aid infection. The two IGS polymorphisms conceivably contribute to different pathogenicity of *C. truncatum* races and are recent evolutionary events..

579. *Unisexual Reproduction - Climbing the Hill, Robertson Effect, without a Partner*. Kevin Roach, Joseph Heitman. Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans is a pathogenic basidiomycetous fungus that >1,000,000 infections and 600,000 deaths annually. Despite the presence and comparable fitness of both mating-types, α isolates predominate (>95 - 99%) in both nature and patients. Long thought to be asexual in many environmental, *C. neoformans* undergoes opposite sex mating (**a**-α) under lab conditions and was recently discovered to undergo a novel form of unisexual mating (α-α). The ability to sexually reproduce without an opposite mating-type partner raises questions about the evolutionary impact of unisexual reproduction on predominantly *MAT*α populations. One such question is the effect of unisexual reproduction on the Hill-Robertson effect, a reduction in effectiveness of selection due to interference in selection between linked loci. In asexually dividing populations a beneficial mutation that occurs on a background with deleterious mutations may be lost due to purifying selection on the background. Likewise, two advantageous mutations segregating on different backgrounds will compete and rise in frequency more slowly unless recombination brings them together on the same background. If *C. neoformans* populations, were limited to only rare opposite mating-type sexual reproduction because of the preponderance of α mating-type, selection would have less opportunity to act on individual alleles and would instead act on whole genotypes. We tested whether unisexual reproduction between *MAT*α individuals reduces linkage disequilibrium between mutations. We show that unisexual reproduction can break linkage between advantageous and deleterious mutations, as well as bring advantageous mutations into linkage together on the same background, increasing the effectiveness of selection. Unisexual reproduction therefor reduces linkage and allows selection to act on individual alleles to reduce the Hill-Robertson effect, thereby increasing the fitness of populations of finite size.

580. Mycobiontal and photobiontal selection of the lichen family Parmeliaceae in Korea. Seol-Hwa Jang, Jung A Kim, Soon-Ok Oh, Sook-Young Park, Jae-Seoun Hur. Korean Lichen Institute, Sunchon National University, Suncheon, Jeonnam, South Korea.

The lichen association is a symbiotic interaction between a mycobiont (fungal partner) and a photobiont (green algal or cyanobacterial partner). The symbiosis could be a major theme in the history of life and an important force driving evolution. However, it is not easy to understand and divided the symbiotic mechanisms that structure the interactions among potential partners. This study examined algal selection among genera and species in the Parmeliaceae family using morphological aspects and internal transcribed spacer (ITS) of the nuclear ribosomal DNA. A total 124 Parmeliaceae lichens were collected in Korea during 2006 to 2011. According to the morphological analysis of lichens, seven genera including *Xanthoparmeliae* spp., *Parmelia* spp., *Parmotrema* spp., *Cetrelia* spp., *Menegazzia* spp., *Nephromopsis* spp., and *Puctelia* spp. dominated, comprising 80.0% of the lichen samples. Based on the internal transcribed spacer (ITS) region sequences from each symbiont, we compared the genetic structure of both mycobionts and photobionts. Phylogenetic reconstructions with maximum likelihood, maximum parsimony and neighbor joining divided the mycobiont and phtobiont data sets into 34 genotypes (F1 to F34) and 15 genotypes (*Trebouxia* sp. 1 to *T.* sp. 9, *T. corticola* 1, *T. corticola* 2, *T. gelatinosa*, *T. incrustata*, *T. jamesii*, and *Asterochloris* sp.), respectively, suggesting evolution of fungal and algal partner may not be equal. The network analysis of the association between fungi and algae showed that most of mycobionts are consistently associated with specific photobiont genotypes including *T.* sp 1, *T.* sp 2, *T.* sp 6, *T. corticola* 1, *T. corticola* 2, *T. gelatinosa*. Therefore, we conclude that fungal selectivity for algal photobionts could be a major factor to determine the availability of symbiotic partnerships.

581. Molecular detection of rust fungi on turfgrass. Brijesh Karakkat, Vonte Jackson, Paul Koch. Plant Pathology, University of Wisconsin, Madison, WI.

Rust is one of the main diseases of turfgrass in Wisconsin and across the country. Historically, the two primary rust diseases on cool-season turfgrasses have been crown rust [CR] (*Puccinia coronata)* and stem rust [SR] *(Puccinia graminis).* Increasing rust severity has been documented in the past 10 years, and recent research indicates it may be due to increasing activity of CR on a wider array of turfgrass species. We are developing a Quantitative polymerase chain reaction (QPCR) detection method to boost this screening process so that the identification is more reliable and faster than only microscopic observation of urediniospores and teliospores. Initial attempts at extraction of genomic DNA from rust-infected leaf samples gave only a few positive results in the traditional PCR mainly because genomic DNA from uredinia were only present in trace amounts. However, more consistent positive results were obtained when QPCR was employed with the same genomic DNA using sequence specific fluorescent probes of *P. coronata* and *P. graminis*. By conducting this study, we aim to test over 400 rust samples collected from different turf plots from around the Midwest in 2013 and 2014 and report the percentage of each *Puccinia* species observed.

582. Evolution of hybrid *Epichloë* species: Independent hybridization or horizontal transmission. Carolyn A. Young[1], Nikki D. Charlton[1], Christopher L. Schardl[2]. 1) Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK; 2) Plant Pathology Department, University of Kentucky, Lexington, KY.

Cool-season grasses from the subfamily Pooideae are widely distributed, inhabiting many different ecological niches. For some, their success is attributed to the dominant systemic symbiotic partner, an endophytic *Epichloë* species (Hypocreales; Clavicipitaceae). *Epichloë* species can be sexual (usually nonhybrid) or asexual (hybrid or nonhybrid) and exhibit different transmission strategies (horizontal, vertical or both). Molecular analyses of the symbiont genetic traits among and between host populations allowed us to explore resident endophyte incidence and diversity in host species across multiple grass tribes. *Epichloë* species within a host and between host populations were found to exhibit considerable genotypic and chemotypic diversity within the bioactive alkaloid biosynthetic pathways. Phylogenetic analyses of intron-rich housekeeping genes (*tefA* and *tubB*) and mating-type were used to infer hybrid and nonhybrid origins. Inheritance patterns of the mating-type idiomorphs signified hybrid species that have arisen from multiple hybridizations of the same parental species but with different chemotypes, giving rise to added chemotypic diversity. Multiple *Epichloë* taxa or single taxa with multiple chemotypes were found to associate independently with a single host species. Those hybrid *Epichloë* species that have been found in diverse hosts will be examined to distinguish whether they may have jumped between those hosts or, alternatively, arisen from independent hybridization events.

583. Priority effects during fungal community establishment in beech wood. Jennifer Hiscox[1], Melanie Savoury[1], Sarah Johnston[1], Carsten Müller[1], Björn Lindahl[2], Hilary Rogers[1], Lynne Boddy[1]. 1) Cardiff University, School of Biosciences, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX; 2) Swedish University of Agricultural Sciences, Dept. of Soil and Environment, Box 7014, SE-750 07 Uppsala, Sweden.

Fungal communities in decomposing wood change with time, often beginning with relatively ruderal species and those establishing from propagules latently present in functional sapwood. These are subsequently replaced by other species, particularly combative basidiomycetes. At later stages fungi with other characteristics may appear, e.g. those tolerant of nutrient stress, or able to obtain nutrition from the mycelia already present, or from by-products of their activity. Fruit body surveys hint that some fungi are associated with specific predecessors, i.e. they are more frequently found fruiting after certain species than after others. However, fruit bodies are a poor surrogate for active mycelium, and it has not been clearly determined whether the fungal species that arrive first dictate the subsequent pathway of community development, i.e. whether there is a priority effect at the species level. We used traditional culture-based techniques coupled with sequencing of amplified genetic markers to profile the communities in beech (*Fagus sylvatica*) disks that had been pre-colonised separately with nine species from various stages of fungal succession. Clear differences in community composition were evident following pre-colonisation by different species, with three distinct successor communities identified, indicating that individual species may have pivotal effects in driving assembly history. However, priority effects were strongly affected by forest site - the pattern was not the same on each one of the eight mixed deciduous forest experimental sites, which were geographically separated by between 0.25 and 105 miles. Priority effects may depend on the available air spora and soil-borne spores and mycelia, and may be linked to biochemical alteration of the resource and combative ability of the predecessor. Also, there was a strong correlation between fungal community structure and wood pH.

584. A highly diverse clade of melanized fungi associated with leaves and trichomes of the endemic tree *Metrosideros polymorpha* at high elevation sites in Hawai'i. Naupaka Zimmerman[1], A. Elizabeth Arnold[1,2], Peter Vitousek[3]. 1) School of Plant Sciences, University of Arizona, Tucson, AZ; 2) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 3) Department of Biology, Stanford University, Stanford, CA.

The Hawaiian Islands are among the most isolated landmasses on earth, separated by thousands of miles of ocean from the nearest continent. The youngest island in the Hawaiian chain, the Island of Hawai'i, is home to multiple volcanoes reaching 4000 m in elevation. This combination of isolation and high elevation creates a distinctive environment in which few plant species establish and grow. Of the hundreds of endemic tree species in the archipelago, only one grows at these highest elevation sites: *Metrosideros polymorpha* (Myrtaceae). As part of a culture-free study focused on the biogeography of foliar fungal endophyte communities, ITS1 pyrosequencing revealed unexpectedly high genetic diversity in a novel clade that was found almost exclusively at high elevation sites (1800m and 2400m elevation). Across a broad elevation gradient (100m to 2400m), endophyte species richness was generally higher at lower elevations, except for the portion of diversity represented by the novel clade: diversity in that lineage yields exceptionally high diversity at the highest sites. Well-supported phylogenetic reconstructions using Bayesian and Maximum Likelihood approaches place the clade in the Teratosphaeriaceae (Dothideomycetes). Subsequent culture-based investigations revealed that members of this clade live in close association with leaf trichomes of their hosts and grow exceptionally slowly in culture. Analysis at the population level provides insight

into origins and distributions across the highest points on volcanoes of the Hawaiian Islands, and hypotheses regarding ecological modes in these unusual environments.

585. Identification of candidate effectors in the poplar rust fungus *Melampsora larici-populina* through a population genomics approach. Antoine Persoons[1,2], Fabien Halkett[1,2], Stephane De Mita[1,2], Sebastien Duplessis[1,2]. 1) INRA, Unité Mixte de Recherche 1136 INRA/Université de Lorraine, Interactions Arbres-Microorganismes, 54280 Champenoux, France; 2) Université de Lorraine, Unité Mixte de Recherche 1136 INRA/Université de Lorraine IAM, 54506 Vandoeuvre-lès-Nancy Cedex, France.

The outcome of host-pathogen interactions depends on a complex molecular dialogue between the protagonists. Effectors released by the pathogen are critical for the success of infection, as they interfere with host metabolism, signaling and defense responses and allow expression of the disease. Effector proteins reported so far in rust fungi exhibit common features (e.g. secreted, small, cysteine-rich) and candidate effectors most likely reside among fungal secreted proteins. *Melampsora larici-populina* is a fungal pathogen responsible for the foliar rust disease on poplar trees, causing severe damage in plantations. Almost all the resistances (R) released so far have been overcome, with the latest major breakdown event in 1994 (R7). The genome of the virulent 7 isolate 98AG31 has been sequenced using a whole genome shotgun strategy, revealing a large genome of 101 megabases containing 16,399 predicted genes including 1184 small secreted proteins. A population genetics study based on 600 isolates was performed to finely determine the impact of this breakdown on the demographic history of *M. larici-populina.* The genomes of 80 poplar rust isolates, distributed among three genetic groups, were sequenced using Illumina technology to understand the effect of the R7 breakdown at the genetic scale. More than 300,000 polymorphic sites (SNPs) were uncovered across isolates, indicating a remarkable level of polymorphism. In order to understand the emergence of the virulence 7, we performed a genome scan analysis based on SNP data using differentiation and selection indices, taking into account the demographic history. We found several genomic regions related to the virulence 7 that bear genes encoding small secreted proteins. This study demonstrates the benefit of population genomics in the search for candidate effector genes. Functional validation of the most promising candidates is underway. AP is supported by the Lab of excellence ARBRE (ANR-11-LABX-0002-01) and the SFP.

586. DMI resistance in *Zymoseptoria tritici*: a history of gradual molecular evolution. Patrick Brunner, Bruce McDonald. Integrative Biology Zurich, ETH Zurich, Zurich, Switzerland.

Fungicide resistance in crop pathogens is a global threat to food production but surprisingly little is known about the evolutionary processes associated with the emergence and spread of fungicide resistance. We evaluated early, mid and late stages in the evolution of fungicide resistance using the wheat pathogen *Zymoseptoria tritici*, taking advantage of a global isolate collection spanning +20 years. We analyzed sequences of the nuclear *CYP51* gene implicated in multiple-mutation resistance to azole fungicides. Results are discussed with particular regard to the roles of selection and intragenic recombination as driving evolutionary forces.

587. Evolutionary constrains of host specificity in the smut fungus *Microbotryum*. Britta Bueker[1,2], Michael Hood[2], Dominik Begerow[1]. 1) Ruhr-Universität Bochum, AG Geobotanik, Universitaetsstr. 150, 44780 Bochum, Germany; 2) Amherst College, Biology Department, 01002 Amherst, MA, USA.

In fungal pathogens, the occurrence of interspecific hybridization is often linked to host species´ distribution and characteristics. Therefore, analysis of the genetics underlying host specialization is a crucial factor for understanding pathogen´s evolution and the involved mechanisms. In the current study we use the basidiomycetes smut fungus *Microbotryum* – a species complex with independent evolutionary lineages that typically specialize to a given host plant species – to study genetic determinants of hybridization and host specialization. To do so, we are analyzing and comparing the genomes of the two *Microbotryum* species, *M. lychnidis-dioiceae* and *M. silenes-acaulis* – both species that are well adapted to distinct host environments. The use of hybrids and selective infection experiments allows us to assess genomic constraints on hybrid viability and to isolate the effect of the mating type chromosomes in relation to host adaptation. The results suggest that loci involved in the disease interactions may be associated with the mating type chromosomes. Furthermore, by focusing on the occurrence of genes underlying positive selection in F1 hybrids and selected backcrosses, potential candidate genes that play a crucial role in infection and virulence can be described. Thus, the application of selective infection experiments in combination with genomic analysis is a feasible approach to elucidate the evolutionary forces of host specificity in the *Microbotryum* pathogen complex..

588. For form and function: developing a new genetic transformation system to correlate the molecular evolution of a putative 'morphogenetic toolkit' with the emergence of diverse phenotypes in Chytridiomycota. Jaclyn Dee, Mary Berbee. Botany, University of British Columbia, Vancouver, BC, Canada.

Like their relatives, the Dikarya, zoosporic Chytridiomycota have evolved an array of both unicellular and hyphal thallus forms, enabling them to occupy a wide variety of ecological niches. Our research seeks to uncover how gene evolution contributed to the stunning diversity of fungal form and function on Earth. We propose that the convergent origins of hyphae in Dikarya and Chytridiomycota may indicate the presence of an ancient 'morphogenetic toolkit' for filamentous growth, in their shared ancestors. Gene families shared by lineages that diverged early from one another and are known to guide fungal morphogenesis, are candidate components of this putative molecular toolkit. In collaboration with the Joint Genome Institute Community Sequencing Project, we obtained genome sequences for two zygomycetes and one hyphal species of Chytridiomycota. With these new data, we performed comparative genomic and subsequent phylogenetic analyses of septins from a representative and diverse sampling of species across the fungal tree. Our analyses suggested that septins, which mediate septation and polarized growth, radiated extensively prior to the divergence of the ancestors of Dikarya and Chytridiomycota, thus implying that septins are good, candidate morphogenetic toolkit components. Connecting septin gene evolution to its role in molding cell shapes is already possible in model fungi, however, in Chytridiomycota, it is impeded by the lack of a reliable genetic transformation system. Using a biolistic genetic transformation protocol, we generated cultures of *Chytriomyces hyalinus* resistant to hygromycin B. We have re-amplified the hygromycin B-resistance gene construct from some of our resistant cultures and hope to

confirm genomic integration of the construct with a Southern blot. Continuing to develop this transformation system is the first step in establishing routine methods that will allow us to link gene evolution and protein function and localization.

589. Genome analyses reveals evolution towards homothallism in *Leptographium sensu lato*. T. A. Duong[1], Z. W. De Beer[2], M. J. Wingfield[1], B. D. Wingfield[1]. 1) Dept of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; 2) Dept of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

Species of *Leptographium sensu lato* (Ophiostomatales, Ascomycetes) are sap-stain fungi primarily vectored by bark beetles infesting conifers. Most species in this genus are known for their capability of causing blue stain in freshly exposed timber, but some species are considered tree pathogens. Of the more than 90 species currently accommodated in the genus, sexual states have been observed for just over 30 species, traditionally treated in the 'teleomorph' genus *Grosmannia*. The remaining species are known only by their asexual morphs and have been treated in *Leptographium*. A recent study using mating type markers revealed that only three of 40 investigated species in this genus were homothallic, whereas the majority species were heterothallic. To date, the MAT loci have been characterized in 11 species in the genus, all of which represent a heterothallic mating system. The aims of this study were to identify the MAT loci in homothallic species and to investigate the evolution of sexual mating systems in *Leptographium sensu lato*. We sequenced genomes of selected homothallic and heterothallic species and identified the MAT loci from these sequences. The results showed that the structure of MAT idiomorphs in heterothallic species were similar to those of other heterothallic species in this genus. While the presence of both MAT idiomorphs in their genomes is a typical characteristic of homothallic ascomycetes, homothallic species in the *Leptographium sensu lato* had unusual organizations of MAT loci, in which the *MAT1-1* and *MAT1-2* idiomorphs resided at different locations in the genome. The unique organization of the MAT loci in homothallic species suggests that these species obtained their homothallic lifestyle by acquiring opposite MAT idiomorphs into their ancestral heterothallic genome. Phylogenomic analyses of shared single copy orthologous genes suggested that the evolution towards homothallism has occurred on multiple occasions in the evolution of this genus.

590. Variability in centromeric sequences of *Neurospora crassa*. Steven Friedman, Pallavi A. Phatale, Jonnathan M. Galazka, Kristina M. Smith, Michael Freitag. Biochemistry & Biophysics, Oregon State University, Corvallis, OR.

Early studies on the centromeres of budding yeast suggested the existence of “centromere consensus sequences,” similar to telomeric TTAGGG repeats or specific transcription factor binding sites. Today, centromere researchers agree that centromeres are epigenetically defined, specifically by the presence of a centromere-specific histone H3 variant, CENP-A (called CenH3 in filamentous fungi). However, many questions about the significance of DNA composition and conformation at *bona fide* centromeric loci remain unanswered. How can centromere sequences be highly variable and apparently undergo rapid evolution compared to most non-centromeric loci without affecting the functioning of the centromere and kinetochore? The balance of homologous recombination versus gene conversion has recently been addressed [1] and many studies have found suppressed recombination within centromeres and pericentric heterochromatin by application of single marker genes, yet mechanisms must exist to allow diversity at this essential chromosomal locus. We addressed this in *Neurospora crassa* by examining centromere sequence dynamics between two strains from a well-defined lineage of laboratory strains that have been used for more than 50 years. These strains contain distinct centromere sequences, some are almost identical, some share more than 75% of their sequence, and some are completely different. We analyzed complete centromeric DNA sequence by high-throughput sequencing both before and after crosses to determine the extent and nature of recombination and gene conversion in centromeric DNA. Based on preliminary data, and similar to previous results from yeast and maize, we show that gene conversion and not homologous recombination, is prevalent in centromeric DNA.

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591. Population genomics of the wheat pathogen *Zymoseptoria tritici*. A. Genissel, L. Gout. Campus AgroParisTech, INRA UMR1290, Thiverval Grignon, France.

Understanding how species respond to environmental changes is a fundamental question in Biology. Standing genetic variation clearly contributes to adaptation. We are interested in tackling this question by looking at standing genetic variation in fungi populations present in contrasted environments. We sequenced 32 isolates of *Zymoseptoria tritici* (pathogen of wheat) collected in the North and South of France using Illumina paired-end sequencing. The goal of this work was to provide an in depth description of natural genetic variation in the fungal populations to further assess its role in adaptation. Main results first describe the nature and distribution of variants along the genome and an elevated amount of variants among the 13 core chromosomes, which places *Zymoseptoria tritici* next to the most diverse eukaryotic species. Second we observe a surprisingly high recombination rate that emphasizes the prominent role of sexual reproduction in the life cycle of the fungus.Third strong Fst values between the North and the South populations are found at many sites throughout the genome. *In vitro* growth assays at different temperatures evidenced phenotypic plasticity among the isolates and we found that the genotype, temperature, genotype-by-temperature and latitude effects were all significantly contributing to the total phenotypic variance. These results contribute to the understanding of the role of standing genetic variation in adaptation to environmental changes.

592. The genetic architecture of reproductive isolation between wild and domesticated populations of the apple scab agent, *Venturia inaequalis*. C. Lemaire[1], M. De Gracia[1], C. Roux[2], C. Fraïsse[2], J. Amselem[3], J. Gouzy[4], T. Leroy[1], M. Cascales[1], N. Bierne[2], B. Le Cam[1], C. Lemaire[1]. 1) IRHS, INRA, Université of Angers, Agrocampus ouest, Beaucouzé, France; 2) ISEM UMR 5554 CNRS, Université Montpellier 2, 34200 Sète, France; 3) URGI,Unité de Recherche Génomique - Info, UR1164, INRA, Versailles, France; 4) LIPM UMR 441 INRA, CNRS Castanet-Tolosan F-31326, France.

While domestication of many plants and animals is well studied, the impact of such rapid evolution on the pathogens from wild to cultivated hosts remains largely unknown. Indeed, does the rapid evolution of host drive the evolution of pathogen toward another adaptive

optimum? Under this scenario one expects higher rates of genetic sweeps in population on domestic hosts than in ones on anthropized environments. However, as domesticated species have expanded their host range far from their wild ancestors, divergence followed by secondary contacts may also occur making difficult the identification of genes involved in adaptation to agro-ecosystems. Here we present a population genomics study of the impact of domestication of apple tree on the agent of the apple scab, the ascomycete *Venturia inaequalis*. Apple scab, the main disease of apple trees, was present on wild ancestors of apple trees before domestication (*i.e* 9000 years ago) in Central Asia. Populations of *Venturia inaequalis* sampled in moutain forests of Kazakhstan are genetically and phenotypically different from those sampled in plains on both wild and domestic apple trees. Here, we analyzed nucleotide polymorphisms on 40 kazakh strains : 20 sampled in wild forests and 20 in anthropized area. Demographic inferences from site frequency spectra derived from *ca.* 100,000 synonymous SNPs showed the best evolutionary scenario to be a recent (less than 100 years) secondary contact with heterogeneous migration among loci revealing the occurrence of semi-pervasive barriers to gene flow. In addition, cline analyses showed that *i)* it is possible to disentangle genetic barriers to gene flow caused by local adaptations from genetic incompatibilities revealed by secondary contacts and *ii)* that genomes of domestic strains are invading the wild population.

593. The evolution of assortative mating in experimental populations of fission yeast. Bart Nieuwenhuis, Sergio Tusso Gómez, Hanna Johannesson, Simone Immler. Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden.

Adaptation to local ecological environments requires association of locally beneficial genes. Recombination quickly leads to loss of association between beneficial genes, when mating occurs with locally not fit individuals. To overcome the break-up of locally fit genes, mating between like individuals should be favored by selection, as it keeps beneficial genes together. However, for assortative mating to arise, like-individuals need to evolve recognition mechanisms which need to become associated with ecological-adaptation genes. To test how local adaptation can occur in the presence of recombination, we performed evolution experiments with fission yeast (*Schizosaccharomyces pombe*) in which we used settling speed as ecological trait. First, we performed artificial selection for settling speed to generate diversity, by shortly spinning down cells at 100x*g* and transferring cells from the bottom (fast-settling) and from the surface (slow-settling). After ~500 asexual generations strong divergence for settling speed had evolved, which was associated with phenotypic changes in cell size and shape. Whole genome re-sequencing was performed to analyze the genetic basis of these changes.

The evolved strains were then mated with each other to generate a genetically variable population from which 20 evolving sexual lines were started. In this second ongoing experiment, disruptive selection for fast and slow settling is applied to the sexually reproducing population. For divergent adaptation to occur, assortative mating and eventually reproductive isolation are expected to evolve. Fitness and phenotypic description after 20 sexual generations will be presented.

594. Mitochondrial-nuclear co-evolution in *Schizophyllum commune*. Kristiina Nygren[1,2], Duur Aanen[1]. 1) Laboratory of Genetics, Wageningen University, Wageningen, Netherlands; 2) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.

Genetic variation in mitochondrial genomes has traditionally been believed to mainly accumulate neutrally. This view is currently being modified as studies in a variety of organisms suggest that cytoplasmic genomes evolve by adaptive evolution. In both plants, animals and fungi, cytoplasm-nuclei interactions have been shown to have significant effects on phenotype. In experiments using the basidiomycete fungus *Schizophyllum commune*, it is a common procedure to replace the mitochondria in naturally occurring strains. Yet, little is known about the extent of mitochondria-nuclei interaction effects in this species and in basidiomycetes in general.

S. commune occurs naturally in all continents, except for Antarctica. Genetic analysis has shown evidence of population structure within the global population but individuals from distant continents are fully interfertile when crossed in the laboratory. We have replaced mitochondria in strains of *S. commune* collected from five continents. Thirteen nuclei and 11 mitochondria were included to create a data set containing 93 monokaryotic nuclei-mitochondria combinations. Preliminary results on a subset of these show significant effects of nuclei-mitochondrial interactions on growth rate. Replacing the original mitochondria sometimes increased the growth rate substantially compared to the naturally occurring combination, even when the new mitochondria originated from another continent. An expanded study, including all 93 monokaryons and three growth conditions is being conducted to further investigate the effects. The included mitochondria are being sequenced, and mitochondria-nuclei combinations showing interesting effects on phenotype will be further investigated for gene expression differentiation.

595. A Genetic Analysis of Optimal and Maximal Growth Temperatures among Mesophilic and Thermophilic Members of the Sordariales. A. Robinson, D.O. Natvig, M.I. Hutchinson. Department of Biology, University of New Mexico, Albuquerque, NM.

Understanding the genetics of temperature responses in filamentous fungi has important implications for ecology, cell biology and industry. We are comparing optimal and maximal growth temperatures for strains from varying latitudes and elevations in an attempt to identify candidate strains for genetic analysis. We are employing the mesophile *Neurospora discreta* and the thermophile *Myceliopthera heterothallica*, both members of the Sordariales with fully sequenced genomes. Race-tube experiments show differences between the optimal growth temperatures (OGT) of *N. discreta* strains from New Mexico and Alaska, as well as *M. heterothallica* strains from Germany and Indiana. These experiments are also providing interesting insight as to whether there is variance in maximal growth temperatures across strains from various geographical locations. We are now attempting to quantify differences in OGT by utilizing ratios of growth rates at two varying temperature ranges. Once a scoring system is established, we hope to determine whether differences in OGT segregate among progeny from crosses of these strains, toward the goal of using genomic approaches to identify the genes responsible.

596. Segregation distortion in the progeny of an interspecific cross between *Fusarium circinatum* and *F. temperatum*: nuclear-cytoplasmic incompatibility and hybrid breakdown. G. Fourie[1], L. De Vos[2], B.D. Wingfield[2], N.A. van der Merwe[2], 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.

Natural selection and divergence due to mutation and drift are major drivers of speciation. However, the emergence of new species is mostly also dependent on reproductive isolation through pre- or post-zygotic mechanisms to prevent the development of hybrids. Although little is known about the nature and function of genes involved in reproductive isolation in fungi, the analysis of hybrid progeny from experimental crosses will likely hold valuable clues regarding molecular mechanisms underpinning the process. For example, hybridization between *Fusarium circinatum* and *F. temperatum* has previously been associated with high levels of genetic transmission ratio distortion (TRD), and it is likely that the genes associated with this phenomenon are also involved in reproductive isolation and ultimately speciation. Since segregation in the *F. circinatum* X *F. temperatum* progeny were skewed towards the maternal parent our hypothesis was that the observed TRD was caused by genetic incompatibilities where the presence of the *F. temperatum* mitochondrion influenced the genetic makeup of the hybrid progeny's nuclear component. To test this hypothesis we identified, characterized and mapped nuclear-encoded mitochondrial genes to the chromosomes of *F. circinatum* and *F. temperatum*. Of the 86 genes identified, 50% were positioned within TRD genomic regions or were located near markers that displayed TRD, which significantly deviated from what was expected purely by chance. Our results thus suggest that the observed TRD can indeed be attributed to limited interaction between products encoded by the nuclear and mitochondrial genomes of the hybrid progeny. Our future research will investigate the extent to which such nuclear-cytoplasmic incompatibility mechanisms could potentially drive the evolution of *F. circinatum*, *F. temperatum* and related fungi.

597. Contemporary fungicide applications sign for selection in *Botrytis cinerea* populations collected in the Champagne vineyard (France). Anne Sophie WALKER[1], Adrien RIEUX[2], Virginie Ravigné[3], Elisabeth Fournier[3]. 1) BIOGER, INRA, THIVERVAL-GRIGON, France; 2) UCL Genetics Institute, London, United-Kingdom; 3) CIRAD, UMR BGPI, F-34398 Montpellier, France.

Populations of fungal pathogens may be subject to many selective pressures in agricultural environments. Among them, fungicides constitute one of the most powerful determinants of population adaptation acting in a short time span. Here, we investigated whether fungicides sprays applied yearly in the Champagne vineyard to control the grey mold causal agent *Botrytis cinerea* could shape population structure and evolution. We carried out a 2-year survey (4 collection dates) on three treated/untreated pairs of plots. We found that fungicides treatments had no or little impact on population subdivision at neutral loci, as well as on diversity or reproduction mode. Nevertheless, we found evidence of stronger genetic drift in some treated plots, consistent with the regular application of fungicides. Moreover, we observed spatial structure in resistance frequency for two loci under contemporary selective pressure, as reflected by cline patterns. At last, using a modeling approach, we estimated fitness costs of resistance to fungicides, responsible for resistance frequency decay during winter. Further work helped estimating parameters of positive selection and migration exerted on *B. cinerea* populations, and disentangling the relative effect of the evolutionary forces at work.

Keywords : *Botrytis cinerea*, population structure, selection, migration, resistance cost, fungicides, diversity, cline, vineyard.

598. Maternal effects vary across sexual reproductive development in *Neurospora crassa*. Kolea Zimmerman[1], Daniel Levitis[2], Anne Pringle[3]. 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Max-Planck Odense Center on the Biodemography of Aging, University of Southern Denmark, Odense M, Denmark; 3) Harvard Forest, Harvard University, Petersham, MA.

A large portion of mortality occurs in the form of offspring inviability or non-development, closely tying mortality to fertility. In this study, we tested the hypothesis that maternal effects on offspring production and quality are greater than paternal effects in both offspring number (fertility) and offspring viability (mortality). We designed a fully crossed reciprocal mating scheme using 11 mat-A and 11 mat-a *Neurospora crassa* strains chosen from a set of 24 mat-A and 24 mat-a strains from North America, the Caribbean, and Africa that were genotyped previously (Ellison, Hall, & Kowbel 2011). Precise genetic distances between mating pairs were calculated to control for the effects of crossing distance on offspring production. We performed reciprocal crosses of all 121 strain pairings and collected data on perithecial production, ascospore (sexual spore) production, and various ascospore characteristics. Mixed effects models of these data show that the female parent accounts for the majority of variation in perithecial production, number of spores produced, and spore germination. Surprisingly, both sexes equally influence the percentage of spores that are pigmented. In this fungus, pigmented spores are viable and unpigmented spores are inviable. These results show that while both parents influence all these traits, maternal influence is strongest on both fertility and mortality traits until the spores are physiologically independent of the maternal cytoplasm within the ascus.

599. The origin, distribution and evolution of Type A trichothecenes in the *Fusarium graminearum* species complex. Amy Kelly[1], H. Corby Kistler[2], Robert Proctor[1], Todd Ward[1]. 1) NCAUR, USDA-ARS, Peoria, IL; 2) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN.

Members of the *Fusarium graminearum* species complex (FGSC) are the major cause of Fusarium Head Blight (FHB) of cereal crops worldwide. FGSC strains typically produce one of three B trichothecenes (3ADON, 15ADON, NIV), which can contaminate grain and have toxic effects in animals and humans. Production of a novel Type A trichothecene (NX-2) by some strains of *F. graminearum* from the US was recently discovered. This unexpected trichothecene diversity is the result of polymorphisms in *TRI1*, a cytochrome P450 enzyme that contributes to structural differences in Type A and Type B trichothecenes. To understand the geographic and phylogenetic distribution of the NX-2 toxin type, we developed and used several DNA sequence-based approaches to screen 1,475 isolates from 22 different countries for the NX-2 allele at *TRI1*. Our data indicate that NX-2 production may currently be limited to *F. graminearum* isolates from Canada and the US (ND, SD, MN, CT), and that the distribution of NX-2 alleles mirrors that of the novel 3ADON population in North

America. To investigate the evolutionary origins of NX-2, we assessed phylogeny and patterns of selective constraint at *TRI1* using orthologous sequences from 40 *Fusarium* species. The *TRI1* phylogeny was strikingly different from the species phylogeny, as *F. graminearum* isolates were not monophyletic. NX-2 *F. graminearum* were derived from a Type B ancestor, but they constituted a highly diverged clade. The ratio of nonsynonymous to synonymous substitution rates (ω) was significantly higher along the branch leading to the NX-2 clade in comparisons with the rest of the phylogeny ($\omega_{NX-2} = 1.17$, $\omega_{Type-B} = 0.05$). This finding is consistent with a substantial relaxation of evolutionary constraint at Tri1 during the evolution of the NX-2 lineage, possibly resulting from a partial loss of function or change in substrate recognition. Our results suggest that alterations to gene function have contributed to the evolution of metabolic diversity in the FGSC.

600. Contributions of vertical descent, horizontal transfer and gene loss to the distribution of mycotoxin biosynthetic gene clusters in *Fusarium*. Robert Proctor[1], Theresa Lee[2], Todd Ward[1], Daren Brown[1]. 1) Bacterial Foodborne Pathogens and Mycology, USDA ARS NCAUR, Peoria, IL; 2) Microbial Safety Team, National Academy of Agricultural Science, Rural Development Administration, Wanju, Republic of Korea.

The genus *Fusarium* produces a diverse array of mycotoxins and other secondary metabolites, but individual species contribute to only a small fraction of this diversity. Here, we employed comparative genomic and phylogenetic analyses to investigate the distribution and evolution of gene clusters responsible for production of four mycotoxin families (fumonisins, fusaric acid, fusarins and trichothecenes) among species of *Fusarium*. The results indicate, not surprisingly, that the presence of a functional biosynthetic gene cluster is the major contributor to whether a species can produce the corresponding mycotoxin(s). The fusarin (*FUS*) cluster is widely but not uniformly distributed among *Fusarium* species, whereas the trichothecene *(TRI*) and fusaric acid (*FUB*) clusters have more limited distributions, and their presence is more uniform within the multispecies lineages in which they occur. The fumonisin (*FUM*) cluster also exhibits a narrow distribution, but its presence within lineages is highly discontinuous. The results also indicate that vertical descent and independent cluster loss in lineages/species have been major contributors to the distribution of mycotoxin biosynthetic gene clusters among fusaria, whereas horizontal transfer (HT) between fusaria has had a more limited impact. We obtained evidence that HT between fusaria re-introduced the *FUS* cluster into a lineage that had lost the cluster relatively recently and introduced the *FUB* and *FUM* clusters into lineages that never had the clusters or had undergone ancient losses of them. Finally, the presence of distantly related homologs of the clusters in other genera of Hypocreales suggests that *Fusarium* acquired the clusters by vertical descent from a hypocrelean ancestor or by HT from another genus rather than by *de novo* cluster assembly in *Fusarium* after it diverged from other fungi.

601. Polymorphism of TRI8 gene among the strains of *Fusarium graminearum* species complex. Chami C. Amarasinghe, W.G. Dilantha Fernando. Department of Plant Science, University of Manitoba, Winnipeg, Canada.

Fusarium head blight (FHB) is an economically important fungal disease in wheat, barley and other small grains throughout the world. Species within the *Fusarium graminearum* species complex (FGSC) are the dominant and most widespread pathogen causing FHB. Trichothecene chemotype variation is one of the key factors that are used to analyse the populations of *Fusarium* species that cause FHB. A better understanding of the DNA sequence variation in trichothecene biosynthesis genes and quantitative variation of pathogenicity and DON production in FGSC is important to predict the *Fusarium* population dynamics. It has been reported that the differential activity of TRI8 gene is a key determinant of the 3ADON and 15ADON chemotypes in *Fusarium.* TRI8 gene sequences were analysed in sixty five *Fusarium* strains representing different species; *F. graminearum* s.s., *F. asiaticum*, *F. boothii*, *F. meridionale*, *F. cortaderiae* and *F. austroamericanum*. The Bayesian phylogenetic tree represented 3 monophyletic clades each representing 3ADON, 15ADON and NIV trichothecene chemotypes. When compared the TRI8 gene sequences among the strains, unique differences were observed. No predictive intron sites were found in all strains. The pathogenic potential of strains belonging to each species was analyzed by the ability of the disease to spread within the wheat head from a single point of inoculation. All strains caused considerable disease in spikelets both on Roblin (Highly susceptible- HS) and Carberry (Resistant-R) wheat cultivars. The highest mean disease severity was shown by *F. graminearum* s.s. 3ADON strains and *F. asiaticum* 3ADON strains both on HS and R cultivars. The third highest mean disease severity was shown by *F. graminearum* s.s. 15ADON strains followed by *F. austroamericanum* NIV strains. All other species showed a lower aggressiveness in both HS and R cultivars.

602. Spatio-temporal Dynamics of Fusarium Head Blight and Trichothecene Toxin Types in Canada. Amy Kelly[1], Randall Clear[2], H. Corby Kistler[3], Kerry O'Donnell[1], Susan McCormick[1], Mark Busman[1], Todd Ward[1]. 1) NCAUR, USDA-ARS, Peoria, IL; 2) Canadian Grain Commission, Winnipeg, Canada; 3) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN.

In many parts of the world *Fusarium graminearum* is the primary causal agent of Fusarium head blight (FHB), a disease of cereal crops that adversely affects crop yield, food safety and animal health. We previously demonstrated population structure associated with differences in trichothecene toxin type (15ADON, 3ADON) and documented dramatic changes in pathogen composition in western Canada. To further understand spatio-temporal dynamics of FHB, we evaluated trichothecene diversity and population assignment of 4,086 *F. graminearum* isolates collected across Canada 2005-2007. In addition to 15ADON (62%) and 3ADON types (36%), we identified *F. graminearum* strains capable of producing the recently discovered A-trichothecene, NX-2 (2%) for the first time in Canada. Regional differences in trichothecene frequency were observed, resulting in two longitudinal clines in 3ADON frequency. Temporal patterns also varied regionally, with 3ADON increasing drastically in parts of western Canada, but remaining at relatively low frequency in Ontario and Quebec. Genetic structure remained correlated with toxin type, as the dominant population (NA1) consisted largely of 15ADON isolates, whereas a second population (NA2) consisted largely of 3ADON isolates. However, the rate of population misassignment based on trichothecene type (assignment of 15ADON to NA2 or 3ADON to NA1) increased rapidly, indicating that gene flow is uncoupling trichothecene type from genetic population identity. Gene flow was regionally biased, in that 15ADON misassignment rate was > 3-fold higher than 3ADON misassignment rate in western and Maritime provinces, and in both regions 15ADON misassignment increased over time. In contrast, 3ADON misassignment rate increased annually and was 5-fold higher than 15ADON misassignment rate in eastern

provinces. FHB population diversity is being shaped by dramatically different local selective pressures across Canada. Understanding ecological processes contributing to local selection of different FHB pathogens will aid in the development of novel strategies to control FHB and mycotoxins in cereals.

603. Field populations of *Fusarium graminearum* revealed different recombination pattern. Firas Talas, Bruce A McDonald. Plant Pathology Institute of Integrative Biology, Zurich (IBZ) ETH Zurich, LFW B28 8092 Zurich Switzerland.

Population genomics enable the identification of dynamic forces taking place between different populations as well as within a single population. Further, it helps us to predict genes under selection and hotspots of recombination that arise through local adaptation. *F. graminearum* is a ubiquitous pathogen of cereals causing Fusarium head blight (FHB). Thirteen German-field populations with around 213 single spore isolates were used to conduct this study. Variation in aggressiveness and ability of DON production was estimated within open field conditions over six different environments. We obtain high heritability for mean estimate of FHB and mycotoxin production (0.51, 0.52 respectively). Additionally, fungicide resistance was estimated for each isolate targeting propiconazole, hence we obtained a high heritability (H^2=0.97) with a mean EC_{50} value ranged from 5.4 to 62.2 $mg.l^{-1}$. Sequence analysis revealed that the *CYP51A* and *CYP51C* genes carried several non-synonymous substitutions but the CYP51B protein was not polymorphic. The non-synonymous substitutions were distributed randomly among populations and were not correlated with fungicide sensitivity. However, we tried to acquire more genetic information in order to find out the genetic bases of the resistance range. Thus for each isolate was partially sequenced using the restriction site associated DNA sequencing (RADseq). Using 1129 high quality SNPs we conduct our genomic study. A high genetic diversity was obtained within the populations combined with restricted population differentiation. The pairwise linkage disequilibrium (LD) decayed rapidly within a short physical distance of 200-600 bp. The multilocus LD indicates a sexual recombination in all populations. Different chromosomes showed different level of recombination. Our results defined several genomic regions with a selective sweep pattern. The rapid decay of LD together with the low structured *F. graminearum* populations optimizes all parameters for a powerful statistical genome wide association study.

604. Population genomics and scans for selection in the plant pathogen *Fusarium graminearum*. Christopher Toomajian, Wei Yue, John Leslie. Dept Plant Pathology, Kansas State Univ, Manhattan, KS.

Fusarium graminearum (*Fg*), a haploid filamentous fungus, is a causal agent of Fusarium head blight disease on wheat and barley. The disease leads to decreased yield, economic losses, and the contamination of grain with toxic secondary metabolites, such as trichothecenes. Previous studies have found evidence for frequent outcrossing in this species capable of self-fertilization and genetic clustering into populations associated with genotypes at gene clusters that produce trichothecenes. Others revealed how the genome is functionally organized, yet there is a need for genome-wide sequence-based markers to describe patterns of variation along chromosomes and among geographical regions. The goals of our study are to: 1) analyze population structure of *Fg* isolates from the Americas, comparing genetic cluster membership with collection location and trichothecene genotype; 2) measure patterns of linkage disequilibrium (LD) in order to infer rates of outcrossing and facilitate future GWAS; and 3) scan the genome for patterns consistent with recent positive selection to infer regions involved in genetic adaptation, such as reported population shifts in the US. We have adapted a genotyping by sequencing (GBS) protocol to produce our population genomic dataset. We have performed principal components analysis and used a Bayesian algorithm to characterize population structure. We have computed summaries of LD from multiple populations. Finally, we have utilized genome scans, including ones based on population differentiation and hitchhiking mapping, to screen for loci involved in adaptation. Our GBS data recover thousands of SNPs distributed throughout the genome. We find genetic clustering due to both geographic location and trichothecene genotypes, and also observe intermediate isolates. Patterns of LD are compared to published estimates of genetic recombination frequency, which vary widely along chromosomes. Finally, our genome scans clarify the cause of North American population shifts and identify other regions that may have important roles in *Fg* adaptation. Our results provide new insight into the evolutionary history of *Fg* and set the stage for mapping functional differences that segregate in our sample.

605. *Fusarium oxysporum* f. sp. *canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. Matthew Laurence, Brett Summerell, Edward Liew. The Royal Botanic Gardens and Domain Trust, Sydney, NSW, Australia.

Fusarium wilt is a serious disease of the date palm *Phoenix canariensis*, caused by *Fusarium oxysporum* f. sp. *canariensis* (Foc). A previous study that characterised and compared the genetic diversity of the Australian Foc population with international strains suggested that the Australian population may have had an independent evolutionary origin. The current study compared the species phylogeny of the Australian and international populations and determined that Foc is not monophyletic, separating into three supported lineages across the two phylogenetic species of the Fusarium oxysporum Species Complex. This , thus confirmsing an independent evolutionary origin for Foc. However, phylogenetic analysis of the in contrast to the species phylogeny, putative pathogenicity genes Secreted In Xylem (*SIX*) did not reveal any separation of the Australian and international Foc strains. were largely monophyletic for *F. oxysporum* f. sp. *canariensis* with low nucleotide diversity. Furthermore, there was very low *SIX* sequence diversity within Foc. Horizontal gene transfer is argued to be the most parsimonious explanation for the incongruence between the species and *SIX* gene phylogenies.

606. Genetic isolation between two recently diverged populations of a symbiotic fungus. Sara Branco, John Taylor, Tom Bruns. Plant Microbial Biology, University of California, Berkeley, CA.

Little is currently known about the drivers of fungal population differentiation and subsequent divergence of species, particularly in symbiotic fungi. Here we investigate the population structure and environmental adaptation in *Suillus brevipes* (Peck) Kuntze, a wind-dispersed soil fungus that is symbiotic with pine trees. We assembled and annotated the reference genome for *S. brevipes* and re-sequenced the whole genomes of 28 individuals from coastal and montane sites in California. We detected two highly supported populations with very low divergence and no evidence for migration. However, we detected highly differentiated genomic regions, most notably a region that contains a gene encoding for a membrane Na^+/H^+ exchanger known for enhancing salt tolerance in plants and yeast. Our results are

consistent with a very recent split between the montane and coastal *S. brevipes* populations, with few small genomic regions under positive selection and a pattern of dispersal and/or establishment limitation. This is the first population genomics study comprising a member of the phylum Basidiomycota or of a mycorrhizal fungus, symbiotically associated with vascular plants. It documents the least differentiated fungal populations currently known and identifies a putatively adaptive gene that motivates further functional tests to link genotypes and phenotypes and shed light on the genetic basis of adaptive traits.

607. Sequencing and molecular dissection of *Sk-2* in *Neurospora*. Austin Harvey[1], Jesper Svedberg[2], David Rehard[3], Daren Brown[4], Richard Wilson[5], Patrick Shiu[3], Hanna Johannesson[2], Thomas Hammond[1]. 1) School of Biological Sciences, Illinois State University, Normal, IL; 2) Department of Organismal Biology, Uppsala University, Uppsala, Sweden; 3) Division of Biological Sciences, University of Missouri, Columbia, MO; 4) National Center for Agricultural Utilization Research USDA-ARS, Peoria, IL; 5) Department of Plant Pathology; University of Nebraska, Lincoln, NE.

Neurospora *Spore killer-2* is a selfish meiotic drive element that kills its non-*Sk-2* siblings during sexual sporulation. Although the location of *Sk-2* has been mapped to a large recombination-suppressed region of chromosome III, the physical length and exact borders of the element have remained undefined. We recently assembled a draft *Sk-2* sequence suggesting it to be 2 Mb long with chromosome rearrangements that could account for recombination suppression. In addition, we identified a mutation that completely eliminates spore killing. The corresponding locus, *rfk-1* (*required for killing*), was mapped to the right border of the *Sk-2* element. Experiments to molecularly characterize *rfk-1*, test its function in other fungi, and elucidate the mechanism of spore killing are in progress.

608. Identification of New *Neurospora crassa* Nonself Recognition Loci Using a Comparative Genomics Approach. Jiuhai Zhao[1], Pierre Gladieux[2], Elizabeth Hutchison[3], Joanna Bueche[1], Charles Hall[1], Fanny Perraudeau[1,4], N. Louise Glass[1]. 1) Department of plant and microbial biology, UC berkeley, berkeley, CA; 2) Ecologie Systematique Evolution, Université Paris Sud, Orsay; 3) Biology Department, 1 College Circle SUNY Geneseo, Geneseo, NY 14454; 4) Ecole Polytechnique, Route de Saclay, F-91128 Palaiseau.

Heterokaryon incompatibility is a critical mechanism employed by filamentous fungi to distinguish self from nonself upon fusion of genetically different somatic cells, and is mediated by a programmed cell death pathway. Heterokaryon incompatibility is ubiquitous in filamentous fungi and is genetically controlled by *het* loci, which exhibit characteristic features including balanced polymorphisms and frequently encode proteins with a HET domain. In this study we developed a population genomics approach to identify all *het* loci in the *Neurospora crassa* genome using next-generation genome sequencing data from *N. crassa* Louisiana population strains to search genes showing features typical of *het* loci. Using this approach, we identified about 30 *het* locus candidates, and all known *het* loci were present in our dataset. Further, we showed that one of these candidates functions as a new *het* locusand has three alleles in population, two of which contain a deletion of the HET domain. All three alleles function in *Neurospora crassa* heterokaryon incompatibility.

609. QTL Mapping Reveals Novel Fungicide Resistance Genes in the Plant Pathogenic Fungus *Zymoseptoria tritici*. Mark Lendenmann, Daniel Croll, Ethan Stewart, Bruce McDonald. Institute of Integrative Biology, Plant Pathology, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland.

We used quantitative trait locus (QTL) mapping to identify genes affecting fungicide sensitivity in two crosses of the wheat pathogen *Zymoseptoria tritici*. Restriction site associated DNA sequencing (RADseq) was used to genotype 263 (cross 1) and 261 (cross 2) progeny at ~8500 single nucleotide polymorphisms (SNP) and construct two dense linkage maps. Fungicide resistance was assessed using high-throughput digital image analysis of colonies growing on Petri dishes with or without the azole fungicide propiconazole. We identified three QTLs for fungicide resistance, including two that contained novel fungicide resistance genes. One of these two QTLs contained only 16 annotated genes, among which four most likely candidates were identified. The third QTL contained *ERG6*, encoding a protein involved in ergosterol biosynthesis. Because propiconazole impairs ergosterol biosynthesis, we believe this gene is the source of the mapped variance in this QTL. QTLs affecting colony growth included *CYP51*, encoding the target protein of azole fungicides, as well as *PKS1*, a gene affecting melanization in *Z tritici*. *PKS1* showed compelling evidence for pleiotropy, with a rare segregating allele that simultaneously increased melanization, decreased growth rate and increased propiconazole resistance. This study resolved the genetic architecture of an important agricultural trait and led to identification of novel genes that are likely to affect azole resistance in *Z. tritici*.

610. Microsatellite characterization of *Colletotrichum fioriniae*. Nicholas Pun, Rytas Vilgalys, Jim Clark, Lindsey Becker, Maria-Soledad Benitez, Michelle Hersh. Duke, Durham, NC.

Fungal pathogens greatly influence forest community dynamics and are often implicated in regulating host abundance. Previous studies have shown the importance of *Colletorichum fioriniae* as a widespread forest pathogen able to infect a large number of hosts. The ability for *C. fioriniae* to infect many different hosts could be explained by many cases of local adaptation and a poor understanding of the diversity present in the *C. fioriniae* species complex. In order to further elucidate the *C. fioriniae* species complex relationships, microsatellites were designed *in silico* based on the *C. fioriniae* genome sequence. These regions were then tested on a set of isolates from a variety of hosts and several forest locations. The variability of certain regions suggests that the diversity within the *C. fioriniae* complex may be larger than previously suggested through multi-locus genotyping. These results indicate that additional sampling and possible genome comparisons between various *C. fioriniae* of different hosts and geographical locations may reveal even greater differences.

611. Pseudogenization in pathogenic fungi with different host plants and lifestyles might reflect their evolutionary past. Ali H Bahkali[1], A van der Burgt[4], M. Karimi Jashni[3], Pierre J. G. M. de Wit[2]. 1) Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia; 2) Laboratory of Phytopathology, Wageningen University and Research Centre, Wageningen, the Netherlands; 3) Department of Plant Pathology, Tarbiat Modares University, Tehran, Iran; 4) Applied Bioinformatics, Plant Research International, Wageningen University and Research Centre, Wageningen, the Netherlands.

Pseudogenes are genes with significant homology to functional genes, but contain disruptive mutations (DMs) leading to the production

of non- or partially functional proteins. Little is known about pseudogenization in pathogenic fungi with different lifestyles. Here, we report the identification of DMs causing pseudogenes in the genomes of the fungal plant pathogens *Botrytis cinerea*,*Cladosporium fulvum*, *Dothistroma septosporum*, *Mycosphaerella fijiensis*, *Verticillium dahliae* and *Zymoseptoria tritici*. In these fungi, we identified 1740 gene models containing 2795 DMs obtained by an alignment-based gene prediction method. The contribution of sequencing errors to DMs was minimized by analyses of resequenced genomes to obtain a refined dataset of 924 gene models containing 1666 true DMs. The frequency of pseudogenes varied from 1% to 5% in the gene catalogues of these fungi, being the highest in the asexually reproducing fungus *C. fulvum* (4.9%), followed by *D. septosporum* (2.4%) and *V. dahliae* (2.1%). The majority of pseudogenes do not represent recent gene duplications, but members of multi-gene families and unitary genes. In general, there was no bias for pseudogenization of specific genes in the six fungi. Single exceptions were those encoding secreted proteins, including proteases, which appeared more frequently pseudogenized in *C. fulvum* than in *D. septosporum*. Most pseudogenes present in these two phylogenetically closely related fungi are not shared, suggesting that they are related to adaptation to a different host (tomato versus pine) and lifestyle (biotroph versus hemibiotroph).

612. Genealogical Concordance Phylogenetic Species Recognition in the *Fusarium oxysporum* Species Complex. Matthew Laurence[1], Brett Summerell[1], Lester Burgess[2], Edward Liew[1]. 1) The Royal Botanic Gardens and Domain Trust, Sydney, NSW, Australia; 2) Faculty of Agriculture and Environment, The University of Sydney, NSW 2006, Australia.

Fusarium oxysporum is an important plant and human pathogenic ascomycetous group, with near ubiquity in agricultural and non-cultivated ecosystems. Phylogenetic evidence suggests that *F. oxysporum* is a complex of multiple morphologically cryptic species. Species boundaries and limits of genetic exchange within this complex are poorly defined, largely due to the absence of a sexual state and the paucity of morphological characters. This study determined species boundaries within the *F. oxysporum* species complex using Genealogical Concordance Phylogenetic Species Recognition (GCPSR) with eight protein coding loci. GCPSR criteria were used firstly to identify independent evolutionary lineages, which were subsequently collapsed into phylogenetic species. Seventeen independent evolutionary lineages were initially identified resulting in the recognition of two phylogenetic species. Further evidence supporting this delineation is discussed.

613. Host adaption in the plant pathogenic fungus *Mycosphaerella fijiensis*. Jean Carlier[1], Marie-Françoise Zapater[1], Daniel Bieysse[1], Yanetsy Montero[2], Veronique Roussel[1], Remy Habas[1], Luis Perez-Vicente[2], Catherine Abadie[3], Stephen Wright[4]. 1) CIRAD, UMR BGPI, Montpellier, France; 2) INISAV, Havana, Cuba; 3) CIRAD, UMR BGPI, Guadeloupe, France; 4) University of Toronto, Ontario, Canada.

Plant pathogenic fungi are able to erode quantitative host resistance through changes in aggressiveness, thereby threatening the durability of host resistance. Such erosions are suspected in some areas in the fungus *Mycosphaerella fijiensis*, responsible for a recent and devastating banana pandemic, Black Leaf Streak Disease (BLSD). This study aims to test for the action of host-specific adaptation and to detect host-selected genes in *M. fijiensis*. We collected six samples in Cuba in three locations distributed throughout the banana production zones where resistant cultivars have been used for about 15 years. For each location, about 40 isolates were collected from two banana plots containing either a resistant variety or a susceptible variety located two to 10 km apart. We also included in the study three samples from Honduras where the disease was first introduced in the Latin America- Caribbean area. Some aggressiveness traits of a subsample of about 100 Cuban isolates coming from the three locations and the two cultivars were evaluated under controlled conditions on the same cultivars. A significant host effect was detected in some locations. A genome scan approach was conducted from whole-genome sequencing of pools of individuals (pool-seq). Differentiated genomic regions were detected between pathogen populations from the two cultivars in some locations. Further analyses have been undertaken to elucidate if these differentiated regions are due to either a host selective effect or demographic history.

614. Species composition of the genus *Saprolegnia* and intra-specific variability of the pathogenic oomycete *Saprolegnia parasitica* in fin-fish aquaculture systems. Paul de la Bastide, Cayla Naumann, Wai Lam Leung, William Hintz. Biology Department, Centre for Forest Biology, University of Victoria, Victoria, BC, Canada V8W 3N5.

Saprolegniosis disease is a persistent problem in commercial fish aquaculture that contributes to significant losses in fish production. Despite its widespread occurrence, the genetic diversity of the causal agent(s) in these facilities is poorly understood. To determine the species composition of this genus, we examined sequence variability within the nuclear rDNA ITS region of *Saprolegnia* spp. for a collection of more than 400 isolates from fish aquaculture facilities. Sequence variation supported the designation of species identity based on ITS nucleotide sequence data, when compared to reference sequences. This approach identified at least 5 species in our study and confirmed the validity of using ITS sequence data to assign species identity to unknown isolates from water, fish and fish egg sources. The most common species detected was *Saprolegnia parasitica*, regarded as the primary pathogen of freshwater fish. A subset of the *S. parasitica* isolates, collected over a 21-month period, were evaluated for their intra-specific genetic variability. We used oligonucleotide primers that anneal to short simple repeat (microsatellite) sequences to amplify a set of variable-length products between annealing sites, thus generating a unique profile for each genotype. The presence or absence of these amplified characters was used to compare the *S. parasitica* isolates and evaluate patterns of genetic diversity over time, and among sample locations. Overall, the genetic diversity of *S. parasitica* isolates was determined to be low. However, there was sufficient variability for the isolates collected in the same time intervals to suggest that diversity was the result of a combination of asexual propagation and infrequent sexual reproduction. These genetic markers allow the monitoring of *S. parasitica* genotypes and facilitate the tracking of genotype origin in aquaculture production systems. Overall, our results demonstrate that *S. parasitica* is ubiquitous in aquaculture facilities and the management of saprolegniosis disease will be an ongoing concern in freshwater aquaculture.

615. Differences in fungal endophytes diversity in *citrus sinensis* (Orange) trees irrigated with fresh versus treated waste water. David Ezra, Noa sela, Lior Blank, Guy Haim, Yigal Elad, Maayan Grinberg-Baran. Plant Pathology, ARO The Volcani Ctr, Bet Dagan, Israel.

Water has always been a resource in absence in the Middle East. Water for agriculture, of different origins and quality is used in the

Israeli orchards. One of the major water resources for citrus orchards in Israel is treated waste water. Long term irrigation with waste water influence trees health and appearance. It is clear that the water quality plays a major role in the trees health as well as fruit quality and quantity. Many possible explanations for the trees deterioration have been suggested, among them soil physical character changes, plants root damage and root associated microbial community changes. We suggested another, different approach, claiming that high concentrations of solubles in the treated water are changing the endophytes communities in the trees tissues. These changes cause the loss of beneficial endophytes aiding the trees. We have sampled trees from two orange orchards different only by the water used for their irrigation. Both orchards are of the same citrus variety and stock, planted on the same type of soil and climatic area. Fungal endophytic communities among samples from the two watering were compared using two methods; by isolation of fungi from the different tissues and by molecular methods. Samples were collected from roots, branches and leaves at summer, fall, winter and spring. All samples (72 samples) were used for fungal endophytes isolation and total DNA extraction. Phenotypic characterization and ITS identification were done to isolates from the different samples. Deep sequencing of all samples were performed by amplification of a 28S rRNA LSU sequence (LROR/LR3) of the fungal endophytic communities. Data generated by Illumina (MiSeq technology) was analyzed using MOTHUR software. Ribosomal Database Project (RDP) was used for taxa identification. Differences between the methods used, and among samples and treatments in fungal composition are observed.

616. Assessing microevolution of secondary metabolism: A population genomic study of *Tolypocladium inflatum*. Jon R. Menke[1], Joseph W. Spatafora[2], Kathryn E. Bushley[1]. 1) Department of Plant Biology, University of Minnesota, St. Paul, MN; 2) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

We have initiated a population genomic study to assess fine-scale evolution of genes involved in secondary metabolite biosynthesis in the beetle pathogen *Tolypocladium inflatum*. The draft genome sequence of this entomopathogenic ascomycete revealed its potential to synthesize a diverse array of secondary metabolites beyond the well-described immunosuppressant Cyclosporin A. Using Pac Bio single molecule real time sequencing, we have improved the resolution of the sequenced reference strain *T. inflatum* NRRL 8044. We sequenced and assembled draft genomes of eight additional strains isolated from different hosts and environments using Illumina technology. Assembly, annotation, and comparative genomic analyses of these strains provides an avenue for investigating mechanisms contributing to intraspecific variation in genes and clusters that encode the synthesis of fungal secondary metabolites, including polyketides, nonribosomal peptides, terpenes, and alkaloids. The results of our analyses will shed light on the relative importance of processes such as transposition, selection, recombination, and horizontal gene transfer in the evolution of fungal secondary metabolism and illuminate how they shape the interaction of these fungi with distinct hosts and environments.

617. Characterizing the effects of genetic variation on osmo-signaling dynamics. Selcan Aydin[1], Daniel A. Skelly[1], Mert Aydin[1], Paul M. Magwene[1,2], Nicolas E. Buchler[1,2]. 1) Biology, Duke University, Durham, NC; 2) Duke Center for Systems Biology, Duke University, Durham, NC.

Cells have evolved networks of genes to sense and respond to environmental signals, where the selected phenotype can often be the dynamics of the signaling pathway. Our objective is to understand how signaling dynamics are affected by genetic variation. We are focusing on the high osmolarity glycerol (HOG) MAPK pathway. The HOG pathway senses and responds to hyper-osmotic shock by activating the Hog1 MAP kinase, which regulates both the transcription and activity of glycerol biosynthesis and transport. The HOG pathway dynamic is a classic example of physiological adaptation, where Hog1 is activated upon osmoshock, peaks, and returns to lower activity as glycerol accumulation balances the osmolarity. Yeasts have evolved to colonize diverse environmental niches with different osmolarity profiles and dynamics. To what extent is the genetic variation in diverse strains capable of modifying the phenotypes of osmo-adaptation in yeast? To address this question, we analyzed the variation in HOG pathway gene sequences across a collection of 100 diverse laboratory, clinical and wild *S. cerevisiae* strains. Our analyses show that the variation is concentrated in the osmo-sensor and SLN1 branch of the HOG pathway. Following up, we measured the growth of the 100-strains under osmo-stress. We showed that there is extensive variation in growth rate and osmo-adaptation. Combining the population structure and growth phenotypes of the 100-strain collection we chose a subset of strains to characterize HOG pathway dynamics. We characterized the temporal dynamics of HOG pathway activation via flow cytometry, using the promoter of an osmo-stress response gene *STL1* driving a fluorescent protein. We focused on ∑1278b strain, which showed higher amplitude in reporter gene expression than other strains, including lab strain W303. We showed the variation is inherited by characterizing the progeny of ∑1278b, W303 cross. We are phenotyping more segregants for bulk segregant analysis assisted high throughput sequencing to map quantitative trait loci. Using complementary quantitative and comparative genomics approaches we aim to identify the alleles associated with the variation in HOG pathway dynamics and the underlying molecular mechanisms.

618. A shift from sexual recombination to clonality in a tree pathogen is associated with a host jump. N. Feau[1], M. Sakalidis[1], J. LeBoldus[2], R. Hamelin[1]. 1) Faculty of forestry, UBC, Vancouver, BC, Canada; 2) Department of plant pathology, NDSU, Fargo, ND.

Host jumps are regarded as a major driver of plant pathogen evolution. The Dothideomycete fungus *Mycosphaerella populorum* is endemic throughout the range of eastern cottonwood, *Populus deltoides* and infects woody stems and causes severe cankers on hybrid poplars. The balsam poplar *P. balsamifera* was believed to be non-host for this fungus, but in 2006 a severe outbreak of stem cankers induced by *M. populorum* was observed on a clonal trial of *P. balsamifera* in northern Alberta, suggesting a host shift. Genome analysis of individuals from this outbreak revealed an unusual pattern of diversity compared to populations from the endemic range in eastern and central North-America. We observed long homogeneous regions (60% of the 29Mb genome) alternating with variable regions organized in two to five different haplotype groups. Genome similarity of 98.9% was observed between these individuals and those of the original *M. populorum* population endemic on *P. deltoides*. Using these patterns of divergence and diversity, we inferred that the population infecting *P. balsamifera* has experienced less than 200 sexual generations following the initial recombination of two *M. populorum* individuals. Our model indicates that the split between this population and the endemic population infecting *P. deltoides* that resulted in the host jump occurred less than 1Mya. Furthermore, there was a transposon proliferation in this population as well as the full fixation of the Mat1.1

allele. This suggests that the population infecting *P. balsamifera* moved towards clonality. The origin of fixation of Mat1.1 in the population infecting *P. balsamifera* could be explained by the complete linkage of a cytochrome P450 alkane hydroxylase gene with the Mat1.2 allele. This could have provided the selective driver to a shift from sexual reproduction to clonality in this population. Our results indicate that the successful adaptation of a pathogen population to a new host species was based on a haplotype pool generated from few recombination events, followed by a long history of asexual propagation resulting in isolation from the source population.

619. Phylogenetic and phenotypic characterization of *Ascomycete* yeast beetle endosymbionts. Kayla Muirhead, Youki Sato, Dana Wohlbach. Department of Biology, Dickinson College, Carlisle, PA.

Within the fungal kingdom, symbiotic relationships are widespread and diverse, and include animal and plant parasitism, mutualism, and commensal relationships. Our study focuses on a symbiotic association between detritivorous beetles and their gut-inhabiting *Ascomycete* yeast endosymbionts. In this relationship, the beetles, which have a nutritionally limited diet, presumably acquire vitamins, important nutrients, or essential digestive enzymes produced by the yeasts; similarly, the yeasts may obtain substantial nutritional benefit from the beetle-gut environment. In order to characterize the diversity of *Ascomycete* yeast beetle symbionts and the exact nature of this insect-yeast symbiosis, we sampled beetles from forests in central Pennsylvania. Here, we describe the phylogenetic and phenotypic characterization of yeasts isolated from this habitat.

620. Molecular identification and characterization of wild barley endophytes. Mihwa Yi, Joshua Kaste, Nikki D. Charlton, Will Q. Hendricks, Bradley Hall, Carolyn A. Young. Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK.

Epichloë species (Clavicipitaceae, Ascomycota) are symbiotic fungi well known for their association with many cool-season grasses. Endophyte-host interactions contribute to plant growth promotion, protection from many pathogens and insect pests, and tolerance to drought stress. Resistance to insect herbivores by endophytes associated with wild barley (*Hordeum* spp.) has been previously shown to vary depending on the endophyte-grass-insect combination. We explored the genetic and chemotypic diversity of endophytes isolated from wild barley to provide potential valuable resources for forage improvement. We analyzed wild barley seeds obtained from US Department of Agriculture's National Plant Germplasm System (USDA-NPGS), which have been reported with endophyte incidence. Isolated endophytes from nine viable PI accession lines separated into five distinct genotypes that represented three non-hybrid and two hybrid genotype groups based on sequence analyses of house-keeping, alkaloid biosynthesis and mating type genes. Molecular phylogenetic analyses indicated that all groups had an *E. bromicola* allele and the two hybrid groups had different additional progenitor alleles for *tefA* and *tubB* genes. Five different chemical profiles were predicted based on the presence and absence of the alkaloid genes and these chemical profiles of distinct isolates will be confirmed by mass spectrometry with endophyte-infected plants. The beneficial effects the endophyte provides the host will be tested under various biotic and abiotic stresses.

621. Evidence of geographic structure in panglobal *Ustilago maydis* populations. Iman Sylvain, John Taylor. Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

Ustilago maydis is a hemibiotrophic Basidiomycete pathogen and the causative agent of corn smut disease on maize and wild teosinte. The current distribution of *U. maydis* is panglobal because the fungus has tracked its host around the world. A previous study discovered five genetically distinct clusters of *U. maydis* in the Americas (1), suggesting that although corn is a highly mobile agronomic product, *U. maydis* populations associated with corn may be structured by host, elevation, or geography. In this study we asked whether *U. maydis* populations in the Americas, Europe, and China are geographically structured? We conducted a population genetic analysis of 70 haploid individuals collected from Mexico, Peru, Bolivia, Ecuador, the United States, Italy, Germany, and China. By amplifying 10 microsatellite loci we have identified three major global populations. We believe these populations correspond to the global spread of corn cultivation out of Latin America, into the United States, and later to the Old World. Our microsatellite analysis suggests *U. maydis* populations have recently diverged, and there may have been multiple independent exports of *U. maydis* from North America and Latin America to Europe and Asia. In future work we intend to sequence the genomes of these samples to better understand their phylogeny and biogeography.

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622. Elucidating the genetic basis of virulence in Zymoseptoria tritici - A QTL mapping approach. Ethan Stewart, Mark Lendenmann, Daniel Croll, Bruce McDonald. Plant Pathology, ETH Zurich, Zurich, Switzerland.

Zymoseptoria tritici, causal agent of Septoria tritici blotch, is one of the most damaging fungal pathogens of wheat worldwide. Symptoms include chlorotic and necrotic lesions containing pycnida - the asexual fruiting structures. The reduced photosynthetic capacity caused by leaf lesions can reduce yields by up to 40%. Despite its significance, little is known about the virulence mechanisims in this pathogen. Using genotype and phenotype data, a QTL mapping approach was used to elucidate the genetic basis of virulence using progeny from controlled crosses between parents differing in their virulence phenotypes. Genetic data was generated using a RADseq aproach. To generate accurate phenotype data a high throughput phenotyping method based on automated digital image analysis was used to accurately measure the percentage of leaf area covered by lesions (PLACL) as well as pycnidia size and number. A seedling inoculation assay was conducted using 2 Z. tritici mapping populations on two different winter wheat cultivars.

Pycnidia size and density were found to be quantitative traits that showed a continuous distribution in the progeny. There was a weak correlation between pycnidia density and size and between pycnidia density and PLACL. There were significant differences in PLACL and pycnidia density on resistant and susceptible cultivars. Over 20% of the offspring exhibited significantly different pycnidia density on the two cultivars, consistent with host specialization.

QTLs underling virulence traits have been identified. Results from QTL mapping show a conection between leaf lesions and pycnidia production. Different QTLs are found between different mapping populations and different wheat cultivars. From the QTL mapping results,

candidate genes with a roll in virulence have been identified.

623. Program number not assigned

624. Outcrossing limits propagation of chromosomal inversions in *Neurospora* species. Christopher Hann-Soden, John W. Taylor. Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

At least nine separate transitions from heterothallism to homothallism have occurred within the genus *Neurospora*. In each case the decreased gene flow associated with homothallism may have led to divergence of a new homothallic lineage. Theory predicts that highly inbred lineages should suffer from reduced efficacy of selection due to reduced genetic variation. The consequent degeneration of the genome would then lead to eventual extinction. Yet the abundance of homothallic lineages in groups such as *Neurospora* challenges this theory. We sought to determine the fate of homothallic lineages by comparing the published genomes of homothallic and heterothallic species along with a newly sequenced heterothallic species basal within the genus. Our whole genome approach allowed us to compare the incidence of chromosomal rearrangements as well as sequence variation. We found that heterothallic species from divergent lineages have fewer inversions relative to each other than are found between heterothallic and homothallic species, or between two homothallic species. Because inversions often reduce the fitness of heterozygotes (i.e. display underdominance), and haploid selfing as observed in homothallic species results in entirely homozygous tetrads, inversions that would be purged in heterothallics may persist in homothallics. Additionally, any deleterious inversions may persist in homothallics due to the reduced efficacy of selection on these populations. Together, the lack of selection on underdominant haplotypes and the reduced selection on deleterious haplotypes may lead to the propagation of inversions in homothallic species. Inversion events have been implicated in speciation processes by creating islands of reduced recombination, so this finding could have implications for the speciation of homothallic lineages. The propagation of inversions in a nascent homothallic population may further limit gene flow, accelerating the process of speciation and leading to radiation within homothallic lineages.

Other

625. *RNA-Seq for identifying novel transcripts, alternate splicing and improving current gene annotations in plant pathogen Phytophthora infestans.* Jolly Shrivastava[1], Carol Davis[2], Francine Govers[3], Howard Judelson[2]. 1) Genetics, Genomics and Bioinformatics Program, University of California, Riverside,CA; 2) Plant Pathology department, University of California, Riverside,CA; 3) Laboratory of Phytopathology,Wageningen University, NL-1-6708 PB Wageningen, Netherlands.

Phytophthora infestans is responsible for the late blight diseases of potato and tomato plants. Correct gene models and annotations are important for studying this pathogen. In this study we used RNA-Seq data to modify the current annotations of the *P. infestans* genome. Trinity and PASA were used to update the existing gene models, identify new genes, and identify alternative splicing events. We used genome-guided Trinity to first align the reads to the T30-4 reference genome, and then PASA to align the transcripts obtained by Trinity back to the genome. Out of 18,179 genes currently annotated in the latest public release of the genome, our analysis resulted in the modification of ~10,000 genes with additions of untranslated regions, changes in CDS boundaries, and gene merging. About ~800 genes exhibited alternative splicing, which involved intron retention, alternate start and ending exons and alternate donor/acceptor sites. Nearly 8,000 transcripts did not map to any genes in public annotation, and up to 400 of these appeared to represent new protein-coding genes. This study will help researchers since signal peptides and transcription factor binding sites can be more accurately identified once the 5' ends of genes are known, since protein function can be better-predicted, and since gene expression studies can assign transcripts to the proper open reading frame.

626. FungiDB: An integrated functional genomics database for fungi and oomycetes. Edward Liaw[1], Dai Gu[1], Venkatesh Moktali[1,2], Brett M Tyler[2], Sufen Hu[3], Hamin Wang[3], John Brestelli[3], Debbie Pinney[3], Omar Harb[3], Brian Brunk[3], Jessica C Kissinger[4], David Roos[3], Jason E Stajich[1]. 1) Plant Pathology & Microbiology, Univ California, Riverside, Riverside, CA; 2) 2Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 3) 3Penn Center for Bioinformatics and Department of Biology, University of Pennsylvania, Philadelphia, PA; 4) 4Department of Genetics, Institute of Bioinformatics and Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA.

FungiDB (http://FungiDB.org) is a free online database that enables data mining and analyses of the pan-fungal and oomycete genomic sequences and functional data. This resource was developed in partnership with the Eukaryotic Pathogen Bioinformatics 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. Release 3.2 of FungiDB contains sequence and annotation for over 70 species spanning the Ascomycota, Basidiomycota, zygomycete, and chytrid fungi; including pathogenic species from the *Cryptococcus, Histoplasma,* and *Coccidiodes* genera. 20 Oomycete genomes are also included in this release. In addition to the genomic sequence data and annotation, FungiDB includes transcriptomic data based on 24 RNA sequence and microarray experiments and all expressed sequence tag data from GenBank. All genomes in FungiDB are run through a standard analysis pipeline that generates additional data such as signal peptide and transmembrane domain predictions, GO term and EC number associations and orthology profiles.

The graphical user interface in FungiDB allows users to conduct *in* silico experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in *Candida albicans* that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. FungiDB is supported in part by the Burroughs Wellcome Fund, the Alfred P. Sloan Foundation, USDA NIFA and NIH HHSN272201400030C.

627. Regulation of fungal secondary metabolism by insect competition? A. Regulin[1], N. P. Keller[2], F. Kempken[1]. 1) Abt. für Bot. Genetik und Molekularbiologie, Bot. Institut, Christian-Albrechts-University Kiel, Olshausenstr. 40, 24098 Kiel, Germany; 2) Medical Microbiology and Immunology, Dept of Bacteriology, UW-Madison 1550 Linden Dr., USA.

Fungi produce an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. However, the benefits of secondary metabolites for fungi are often obscure. It is likely that the fungal ability to regulate secondary metabolism reflects an evolutionary adaptation to ensure efficient exploitation of environmental resources and to synthesize secondary metabolite only when the ecological conditions demand it against natural enemies and competitors. However, it should be noted that secondary metabolites are not the sole defense mechanisms of fungi.

We utilized complementary approaches of experimental ecology and functional genomic techniques to enlighten the function of secondary metabolites such as mycotoxins in the chemical defense reactions of insect-fungal interactions. Additionally the influence of these competitors at tropic interactions was investigated.

In our current research the vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are being used as an ecological model system. Microarrays of *Aspergillus nidulans* have been utilized in order to identify the respective fungal up- or down regulated target genes in the interaction with *Drosophila* larvae. Under competing conditions a number of other genes appear to be up-regulated. Thus quantitative RT-PCR was employed to analyze secondary metabolite gene expression after exposed confrontation. In addition fungal single 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*.

628. Transposons as tools: An inducible system for mutagenesis in Ascomycetes. Linda Paun[1], Benjamin Nitsche[2], Arthur Ram[2], Frank Kempken[1]. 1) Botanical Institute, Christian-Albrechts-University, Kiel, Germany; 2) Institute of Biology, University Leiden, The Netherlands.

Transposons are mobile genetic elements found in all eukaryotic genomes. Their impact on gene expression as well as their ability to cause mutations makes them great tools for random mutagenesis (1). In previous work we developed a selectable system for *Vader* transposition in the *Aspergillus niger* strain CBS513.88 and have presented evidence for excision and reintegration at random positions (2,3). To this end, we cloned the non-autonomous transposon *Vader* between the constitutive gpdA promoter and the open reading frame of the *hph* gene. To further improve *Vader* transposition, we have developed an inducible transposition system in the *A. niger* strain N402, that carries a non-functional *tan*1 transposase gene. *Vader* transposition was not observed after transforming the PgpdA-Vader-hph construct into the N402 strain. Hence, the *tan*1 transposase gene from *A. niger* strain CBS513.88 was expressed in N402 under the control of the Tet-on-promoter, which can be activated by doxycycline (DOX) (4). We observed a DOX-dependent *Vader* excision frequency of 10^6 to 10^3 at DOX concentrations of 10 µg/mL and 200 µg/mL, respectively. All colonies analyzed exhibited an excision event at the DNA level and *Vader* footprints were found. This system appears to be a useful tool for inducible transposon mutagenesis in *A. niger*. Additionally, we have introduced *Vader* into *Neurospora crassa*. At current, the *Vader* activity in this heterologous host is under investigation.

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629. Industrial strain construction: Improving the toolbox. Peter van de Vondervoort, Rene Verwaal, Yvonne Arendsen, Noël van Peij. Genetics, DSM Biotechnology Center, Delft, Netherlands.

For the industrial production of enzymes at DSM we use various micro-organisms such as *Aspergillus niger*, *Kluyveromyces lactis* and *Bacillus subtilis*. These hosts have a long history of safe use. For each new enzyme, a production host needs to be developed to produce the protein of interest. The field of molecular biology is developing rapidly. More tools become available to design strains in a rationalized way, build them in a faster way and/or increase the throughput of testing strains. A number of tools developed will be described such as advanced transformation and cloning methods and a Cre-Lox based method for marker removal. We will present an overview of recently developed tools and how they can be used in improved, rationalized and faster strain construction with a higher success rate.

630. Enhanced Hydrolysis of Lignocellulosic Material. Taija Leinonen[1], Kristiina Järvinen[1], Susanna Mäkinen[1], Kari Juntunen[1], Alexandra Komander[2], Kim Langfelder[2], Jari Vehmaanperä[1], Terhi Puranen[1]. 1) Roal Oy, Rajamäki, Finland; 2) AB Enzymes GmbH, Darmstadt, Germany.

Limited resources of fossil fuels have raised a need for using biomass as a renewable and clean source of energy. One promising, alternative technology is the production of biofuels i.e. ethanol from (ligno)cellulosic materials. Enzymatic hydrolysis is considered as potential method for converting cellulosic biomass into fermentable sugars, and efforts have been made to improve the efficiency of the hydrolysis process. An effective enzyme mixture for cellulosic biomass hydrolysis contains at least cellobiohydrolases (CBH), endoglucanases (EG), beta-glucosidases (BG) and xylanases (XYN). The hydrolysis performance could be increased by optimization of the mixture components, or by improving the individual enzyme components in the mixture by protein engineering or enzyme discovery, or by adding novel auxiliary enzymes with additional activities into the mixture. In this work, *Acremonium thermophilum* CBHI, EG_A, EG_B

and *Melanocarpus albomyces* FAE_B were cloned and produced in *Trichoderma reesei*. In the biomass hydrolysis experiments different combinations of enzymes were tested on variety of (ligno)cellulosic substrates. Obtained data indicate that by replacing the existing enzyme components of the mixture with the *A. thermophilum* CBHI, EG_A or EG_B and adding *M. albomyces* FAE_B as auxiliary enzyme into the mixture, have enhancing effect on hydrolysis yield.

631. High-level production of mono-component and enzyme mixtures in *Myceliophthora thermophila*. Cristina Llavata Peris, Jurian Bronkhof, Jurgen Snelders, Hans Visser, Jaap Visser, Jean-Paul Meijnen. Dyadic Netherlands, Wageningen, Netherlands.

The thermophilic fungus *Myceliophthora thermophila* C1 was developed into an efficient and versatile platform for high-level production of industrially relevant enzymes. By means of strain development strategies, such as random mutagenesis and targeted disruptions, we obtained two strain lineages that are being developed and exploited as enzyme production hosts. One strain lineage (HC-strains) is able to produce and secrete high amounts of enzyme mixtures that contain large amounts of (hemi-) cellulases. The other strain lineage (LC-strains) is impaired in its cellulase producing capability, resulting in low background-protein production. For that reason the LC-strains could be very suitable for the production of individual enzymes.

The LC strain has been further developed for high-level production of homologous enzymes. An open reading frame encoding an endoglucanase was introduced at multiple copies by multiple rounds of transformation. After fermentation optimization, protein levels of up to >25 g/L were reached of which ~ 80% was the overexpressed endoglucanase. These results indicate that the LC strain is capable of producing mono-component enzymes at high levels in a relatively pure form.

By transforming the LC strain with selected C1 genes, a wide collection of strains was obtained, each of which produced mainly one enzyme. This has ultimately led to an enzyme library of over 100 functional enzymes of which many have been purified and characterized in detail.

In conclusion, *M. thermophila* C1 was developed into a high-level protein-production platform. The HC strain is successfully applied to produce enzymes for the production of biofuels and biobased-chemicals. The LC strain is being used to produce single enzymes and defined combinations of enzymes. The obtained C1-enzyme library is a rich source for academic and industrial research. The properties of *M. thermophila* C1 make this fungus a highly suitable alternative for traditional fungal protein production hosts.

632. Cytological karyotypes of the two powdery mildew fungi, *Blumeria graminis* f.sp. *hordei* and *Erysiphe pisi*. T. Takaya[1], K. Toyoda[2], M. Taga[1]. 1) Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan; 2) Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan.

Blumeria graminis f.sp. *hordei* (*Bg hordei*) and *Erysiphe pisi* are the important plant pathogens not only in crop production, but also as models for obligate parasites. The genome project of the two species showed peculiar nature of their genomes in terms of genome size (120 Mb and 151 Mb for *Bg hordei* and *E. pisi*), content rates of transposable elements (64 % of the genome in *Bg hordei*), and gene numbers (5,854 in *Bg hordei*). It is intriguing to know how such peculiarity of the genomes is reflected in cytological features of chromosomes and karyotypes. Presently, however, only limited information is available of the genomes of these species from cytological aspects. In this study, we analyzed cytological karyotypes of *Bg hordei* (race1) and *E. pisi* (race1) by combining germ tube burst method (GTBM) and fluorescence microscopic techniques including FISH. In GTBM, conidia were germinated on hydrophobized slides to allow mitosis, and the slides immersed in methanol-acetic acid solution to disrupt germ tubes and spread chromosomes outside cells. For visualizing chromosomes, specimens were stained with DAPI/PI. Summarized results are as follows. (1) Chromosome numbers for the two species were concluded to be $n = 10$ with no minichromosomes being contained in their genomes. (2) The shapes of mitotic chromosomes of the two species were similar to those of other ascomycetes observed with GTBM. (3) FISH revealed one NOR existing in the genomes. Its location on the chromosome was subterminal in *Bg hordei*, and distal in *E. pisi*. Preferential staining of NOR with PI suggested high GC content of this region. Interestingly, the size of NOR in *E. pisi* was almost two-fold larger than that of *Bg hordei*. (4) Constrictions presumably representing centromeres and sister chromatids were discerned in some chromosomes. (5) Relative chromosome length of the largest to the smallest was 3~4:1 in the two species. In conclusion, this study presents reliable and detailed information of the karyotypes and chromosome features for the two species and urges correction of the previous data obtained by conventional cytology.

633. Comparative study of resistance mechanisms to zoxamide and carbendazim in *Botrytis cinerea*. M. Cai[1,2], D. Lin[1], C. Chen[1], X.-L. Liu[1]. 1) Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China; 2) Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, 97331, U.S.A.

Zoxamide and carbendazim are both β-tubulin inhibitors. Unlike resistance to carbendazim, which is of high risk and has developed worldwide in various species, resistance or reduced sensitivity to zoxamide has been rarely reported. The aim of this study was to investigate the molecular basis of resistance to zoxamide in *B. cinerea* and to characterize the resistance molecular differences between the two fungicides. Monitoring of sensitivity in 161 field isolates showed three phenotypes were present, including 26 $S^{zox}S^{car}$ (both sensitive to zoxamide and carbendazim), 88 $S^{zox}R^{car}$ (sensitive to zoxamide but resistant to carbendazim) and 47 $R^{zox}R^{car}$ (both resistant to zoxamide and carbendazim) isolates, but no one was $R^{zox}S^{car}$ (resistant to zoxamide but sensitive to carbendazim). Two $R^{zox}S^{car}$ phenotype isolates were obtained via laboratory selection on zoxamide-amended media; their resistance factors were 13 and 20, and their resistance was stably inherited. The fitness of $R^{zox}S^{car}$ was reduced in terms of mycelial growth rate, sporulation, virulence and sclerotium production. The results suggest that the risk of *B. cinerea* developing resistance to zoxamide is low where carbendazim has not been used. A point mutation of M233I was detected in the β-tubulin of the $R^{zox}S^{car}$ mutants. Moreover, a point mutation (E198A) was found in all $S^{zox}R^{car}$ isolates, and either the point mutation of E198K or F200Y were found in all $R^{zox}R^{car}$ isolates. Molecular docking was used to predict how the mutations

alter the binding of zoxamide and carbendazim with β-tubulin. The results indicated that E198 and F200 may be sites targeted by both zoxamide and carbendazim. The amino acid substitutions at the two sites eliminate hydrogen bonds connecting them with the fungicides. M233 may be a unique target site for zoxamide, which causes the differences between the two fungicides. The docking model showed that the hydrophobic interactions weakened when methionine was mutated to isoleucine at codon 233. M233 may be also very important for the function of β tubulin, based on the fitness penalty observed in the mutants.

634. The shades of ROS: Old problems, new solutions. Robert Marschall, Ulrike Siegmund, Paul Tudzynski. Institute of Plant Biology, University of Münster, Münster, Germany.

Reactive oxygen species (ROS) are produced in all cells that depend on molecular oxygen. They are produced in conserved cellular processes either as byproducts of the cellular respiration in mitochondria or as a support for defense mechanisms, signaling cascades or cell homeostasis. ROS have two diametrically opposed attributes due to their highly damaging potential for DNA and lipids as well as due to their indispensability for signaling and developmental processes.

The major sources of enzymatically produced ROS are NADPH-oxidase complexes (NOX) which generate superoxide. To cope with increasing concentrations of ROS, every organism has evolved scavenging systems (glutathione system/superoxide dismutase) that counteract ROS-producing enzymes and facilitate the detoxification of ROS. Due to the important role of ROS, many studies aim to uncover and understand ROS producing and ROS detoxifying enzymes, signaling pathways and dependent differentiation processes. To characterize the localization and flux of ROS, several tools (NBT, H_2DCFDA) have been established to monitor ROS levels, but few are applicable for fungi, or specifically for a necrotroph like *B. cinerea*.

A new assay based on commercial available fluorescent dyes enables now the detection of O_2^- and H_2O_2 specifically and simultaneously. Moreover, it allows the quantification of secreted and intracellular ROS (Marschall and Tudzynski, 2014).

While the fluorescent-dye-based assay is suitable to monitor the presence of ROS, the redox sensitive RoGFP2 facilitates the visualization of redox state changes (Heller et al., 2012). By generating fusion constructs of the biosensor and signal peptides, it is now possible to consider the effect of chemical agents on the redox state in nearly all compartments. Besides, it can be examined which impact the deletion of subunits of the Nox-complex has on the overall redox state.

Heller et al., (2012) Mol Plant Pathol. 13(8):935-47

Marschall R and Tudzynski P (2014) Fungal Genet Biol.;71:68-75.

635. Systematic genetic characterization of long non-coding RNAs in the model human fungal pathogen *Cryptococcus neoformans*. Youbao Zhao[1], Nadia Chacko[1], Ence Yang[2], James Cai[2], Xiaorong Lin[1]. 1) Biology, Texas A&M University, College Station, TX; 2) Vet School, Texas A&M University, College Station, TX.

Long noncoding RNAs (lncRNAs) are non-protein coding transcripts larger than 200 nucleotides. In mammals, lncRNAs play important roles in a variety of biological processes, such as aging, development, and oncogenesis. Yet, the prevalence and the biological functions of lncRNAs in human fungal pathogens remain unexplored. *Cryptococcus neoformans* is an environmental opportunistic pathogen. This ubiquitous fungus is responsible for one million cases of cryptococcal meningitis and more than 600, 000 deaths annually. Cryptococcal morphogenesis has been a topic of interests for research given its importance in fungal pathogenicity. Our genetic screen led us to the functional identification of an lncRNA (*RZE1*) that coordinates cryptococcal morphogenesis and virulence. We found that *RZE1* exerts its impact on morphogenesis through its control over the morphogenesis master transcription factor Znf2. Interestingly, *RZE1* controls *ZNF2* transcription and likely also the nuclear export of *ZNF2* transcripts. Through reanalyzing the existing RNA-seq data for the clinical strain H99 (serotype A) and our own transcriptome data for the strain XL280 (serotype D), we identified 1,100 lncRNA-like transcripts conserved in this species complex. We are currently utilized deep RNA sequencing to analyze cryptococcal transcriptomes under multiple culturing conditions and developmental stages. We expect to identify lncRNAs that show correlated transcription profiles with genes that encode important virulence factors and morphogens. We expect some of them will be their neighboring genes as lncRNAs generally imping on the expression of adjacent genes. The ongoing systematic genetic analyses of lncRNAs will help us understand coordinated global transcription during cryptococcal infection and development, and point to previously unknown regulatory mechanisms in fungi.

636. Metagenomic mycoflora analysis of Korean traditional wheat *nuruk*. Jyotiranjan Bal[1], S.-H. Yun[1], J.-M. Kim[2], S.-H. Yeo[3], J.-H. Kim[3], K.-Y. Jahng[1], D.-H. Kim[1]. 1) Institute for Molecular Biology and Genetics, Chonbuk National Univ, Jeonju, Jeollabuk-do, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeollabuk-do, South Korea; 3) Fermented Food Science Division, Department of Agrofood Resource, NAAS, RDA, South Korea.

Nuruk, a traditional natural starter is extensively used in the brewing of *Makgeolli*, one of Korea's most popular alcoholic beverages gaining global popularity recently. In the wake of this, the quality of traditional *nuruk*, needs to be enhanced. Due to the limitations of culture dependent identifications of the vast majority of microorganisms present in most environments of earth, metagenomic analysis was performed to characterize the mycoflora and their temporal variations associated with traditional wheat based *nuruk*s fermented at two representative traditional temperature conditions over a span of 30 days. *Nuruk* A was fermented at a constant temperature of 36°C for 30 days and *nuruk* B was fermented at a high initial temperature of 45°C for 10 days followed by 35°C for 20 days. Using a high-throughput pyrosequencing technique with fungal specific primers targeting the internal transcribed spacer 2 region, a total of 73,789 filamentous fungi

sequences were obtained. These sequences were further characterized into 77 genus and 175 species. This analysis also suggested that *nuru*k A consisted of the most diverse mycoflora on the sixth day of *nuruk* fermentation, whereas *nuruk* B had the most diverse mycoflora on tenth day. The mycoflora of *nuruk* A was more diverse than *nuruk* B at almost all timepoints of nuruk fermentation. The analysis suggested a predominance of the genus *Aspergillus* in *nuruk* A and *Rhizomuco*r in *nuruk* B. *Aspergillus tritici*, was the predominant species followed by *Aspergillus oryzae, Lichtheimia corymbifera* and *Aspergillus candidus* at all time points of *nuruk A* fermentation. Metagenomic analysis revealed that both samples A and B varied considerably with respect to the fungal communities over a span of 30 days..

637. Fungal diversity associated to microhabitat variation revealed by 454 pyrosequencing in a semiarid ecosystem in Baja California, Mexico, endemic for Valley Fever. Lluvia Vargas Gastelum[1], Adriana L. Romero-Olivares[4], Ana E. Escalante[5], Carlos Brizuela[2], Axayácatl Rocha-Olivares[3], Meritxell Riquelme[1]. 1) Microbiology, CICESE, Ensenada, Mexico; 2) Computer Sciences, CICESE, Ensenada, Mexico; 3) Biological Oceanography, CICESE, Ensenada, Mexico; 4) Ecology and Evolutionary Biology, University of California-Irvine,California, USA; 5) National Laboratory of Sustainability Sciences, Ecology Institute, UNAM, Mexico.

Fungi play fundamental ecological roles in terrestrial ecosystems. However, their distribution and diversity remain poorly described in natural communities, particularly in arid and semiarid ecosystems. In order to identify environmental factors determining fungal community structure in these systems, we assessed their diversity in conjunction with soil physicochemical characteristics in a semiarid ecosystem in Baja California, Mexico, endemic for Coccidioidomycosis (Valley Fever). Two different microhabitats, burrows (influenced by rodent activity) and surface (topsoil), were compared in winter and summer. For fungal identification, 454 pyrosequencing of the ITS1 region of nuclear ribosomal DNA was used as barcode. A total of 1,940 OTUs were identified from 362,332 ITS1 sequences. Differences in fungal abundance and richness between microhabitats and seasons were identified. Differences in fungal diversity among microhabitats were mainly correlated to significant differences in environmental factors, such as moisture and clay content for surface samples, and temperature and electrical conductivity for burrow samples. Overall, the fungal community structure (dominated by Ascomycota and Basidiomycota) was less variable between seasons in burrow than in surface samples. *Coccidioides* spp. went undetected by pyrosequencing. However, a nested PCR approach revealed its higher prevalence in burrows.

638. A CRISPR/Cas9 system for genetic engineering of filamentous fungi. Christina S Noedvig, Jakob B. Nielsen, Uffe H. Mortensen. DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark.

The harnessing of the prokaryotic and archaeal immune mechanism CRISPR (clustered regularly interspaced short palindromic repeats) as a tool for genetic engineering in eukaryotes, has proved to be a powerful technology. CRISPR/Cas9 introduces specific DNA double strand breaks (DSB) with high precision, which in turn can be employed to efficiently stimulate gene targeting. Consisting of two components, an RNA guided nuclease Cas9 and a chimeric guide RNA (gRNA), a specific DSB can be produced in the host organism. The cleavage target site is determined by 20 base pairs (bp) in the gRNA, and by exchanging those 20 bp, Cas9 can be programmed to target a specific chromosomal location with few constraints. The technology has had a huge impact on genetic engineering of organisms, such as plants or mammalian cells where gene targeting is notoriously inefficient, but has so far not been adapted to filamentous fungi. Low gene targeting frequencies is a common problem when attempting to do gene editing in filamentous fungi. A common strategy to circumvent this problem is to delete or disable one of the key genes in the non-homologous end-joining (NHEJ) pathway to greatly enhance gene-targeting frequencies. However, for fungi where a genetic toolbox is not in place, the initial establishment of genetic markers and NHEJ-deficiency can be laborious. Here we present a CRISPR/Cas9 system adapted for filamentous fungi and show that it can be efficiently used to introduce specific genomic modifications. Considering that the number of fully sequenced fungi is dramatically increasing, and that the vast majority of these fungi does not possess a genetic toolbox, our system will be a highly useful in developing the initial marker- and NHEJ gene mutations to establish such a toolbox. To this end, we have also developed a gRNA design software that facilitates identification of gRNA sequences that can target a desired gene in several different species, hence, reducing the plasmid construction workload. Together, we envision that our tools can be used to rapidly expand the repertoire of fungi where genetic engineering is possible and therefore greatly accelerate the exploration of fungal biology.

639. Efficient gene targeting by generating I-SceI induced double strand breaks in *Trichoderma reesei*. Jean-Paul Ouedraogo[1], Igor Nikolaev[2], Sharief Barends[2], Arthur F.J Ram[1]. 1) Leiden University, Institut of Biology,Sylviusweg 72, 2333 BE Leiden, Netherlands; 2) Dupont Industrial Biosciences, Archimedesweg 30, 2333 CN Leiden, NL.

Targeted integration of expression cassettes for protein production in industrial microorganisms is highly desirable. It ensures reproducible expression levels of the protein of interest among transformants and it prevents potential harmful random integration of the expression cassette in the genome. Targeted integration methods often results in low transformations frequencies. In this study we have expressed the *S. cerevisiae I-SceI* meganuclease in the industrial important filamentous fungus *Trichoderma reesei* to generate double strand breaks at a defined locus in the *T. reesei* genome. We show that the *I-SceI* is active as expression of *I-SceI* enhances dramatically the frequency of recombination after creating a double strand break. The break is efficiently repaired by a recombination event mediated by repetitive sequences surrounding the *I-SceI* restriction sites. As the recombination event also result is the loop out of a selection marker and restoration of GFP fluorescence, the efficiency of recombination can be detected easily. We show that the double strand DNA break mediated by *I-SceI* can also be efficiently repaired by simultaneous transformation of a DNA cassette with homologous flanks, allowing homologous recombination at the locus containing the *I-SceI* restriction sites. We also show that expressing *I-SceI* increases the number of stable transformants obtained in a transformation experiment. Analysis of the transformants obtained via *I-SceI* mediated gene targeting showed that over 65 % of the transformants resulted from a homologous recombination event at the intended locus.

640. Fungal and bacterial transcriptional activity in temperate pine forest soils in response to long-term nitrogen amendment. Cedar N. Hesse, LaVerne A. Gallegos-Graves, Rebecca C. Mueller, Cheryl R. Kuske. Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM.

Soil fungi and bacteria provide integral roles in the decomposition and recycling of carbon, nitrogen, and other nutrients essential to terrestrial ecosystem function. Anthropogenic perturbation of nitrogen balance alters the rates of forest litter decomposition and potentially influences overall soil community metabolism. Using an rRNA depletion approach and Illumina sequencing, we surveyed community mRNA expression directly from the soil in two surface soil horizons subjected to long-term experimental nitrogen addition in Duke Forest, North Carolina, USA. Functional annotation of sequencing reads to PFAM, CAZymes, and Gene Ontology databases allow us to thoroughly explore the genomic basis of biogeochemical pathways mediated by soil fungi and bacteria. Further, we explored how metatranscriptome expression profiles are correlated with additional biotic and abiotic measurements from the same samples including fungal and bacterial taxonomic abundance (rDNA), soil chemistry, and soil enzyme activity. This presentation will highlight the major findings from this study and summarize some analytical challenges associated with metatranscriptomic data.

641. Spatio-temporal dynamics of fungal communities in southern and subarctic boreal forest soil in Finland. Minna Santalahti[1], Hui Sun[1,2], Jukka Pumpanen[2], Kajar Köster[2,3], Frank Berninger[2], Fred Asiegbu[2], Tommaso Raffaello[2], Ari Jumpponen[4], Jussi Heinonsalo[1]. 1) Department of Food and Environmental Sciences, University of Helsinki, Finland; 2) Department of Forest Sciences, University of Helsinki, Finland; 3) Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Estonia; 4) Division of Biology, Kansas State University, USA.

Soil microbes perform essential ecological functions via nutrient cycling and decomposition of organic matter. Fungi are the predominant decomposers in boreal forest and essential for the turnover of soil C and N. Seasonal and spatial dynamics in southern boreal forest and the effect of forest fire on fungal community in subarctic boreal forest were studied by using next-generation 454-pyrosequencing coupled with a high-throughput functional gene microarray GeoChip4.0. The aim of the study was to evaluate fungal diversity, community structure and functions in boreal forests in a south-north transect. In southern and in subarctic boreal forest, 2313 and 2007 OTUs were detected in organic soil horizon, respectively. Seasonally, a dramatic shift in fungal community was observed between growing season and winter time: ectomycorrhizal (ECM) fungi were dominating the community during the growth season but saprotrophs (SAP) were dominant in the winter. Spatially, SAP dominated the upper most raw litter layer whereas ECM dominated deeper layers of soil. In subarctic boreal forest soil, the time after forest fire had significant effects on fungal diversity and structure. The site 2-years after fire had the highest diversity and the diversity decreased with increased time after fire. The functional gene pool correlated positively with OTU richness. Functionally, the youngest (2-year) and the oldest (152 years) sites were more similar with each other than the 60 year old site. Our analyses revealed that the fungal communities are species-rich across the climatic gradient. Despite of observed functional redundancy in the arctic site, the community richness and functional gene diversity correlated positively indicating that higher microbial diversity in soil guarantees higher genetic potential for maintaining crucial biochemical reactions in soil.

642. Oomycete pathogens take advantage of lipid raft and PI3Pon the plant membrane for infection. K. Tao[1,2], B. Tyler[2,3]. 1) Molecular and Cellular Biology, OREGON STATE UNIVERSITY,Corvallis,OR; 2) Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR; 3) Center for Genome Research and Biocomputing, OREGON STATE UNIVERSITY, Corvallis,OR.

Plant pathogens secrete specific effector proteins into host cells to manipulate their immune network. In bacteria, the translocation of thes e effectors utilizes the Type III secretion system. However, in oomycetes and fungal pathogens the mechanism of delivery into the host cell s is being deciphered. In oomycete pathogen, a large superfamily of effectors have been identified by bioinformatics analyses. These effect ors contain a conserved N-terminal RXLR-
dEER motif that plays an important role in effector delivery into host cells. Phosphoinositides are phosphorylated derivatives of phosphatid ylinositol produced by phosphoinositide kinases that are essential in cytoskeletal dynamics, specific membrane trafficking, and signaling ev ents through binding of specific activator proteins. Recently, several studies demonstrated that oomycete and fungal effectors may enter int o host cells through binding to phosphatidylinositol-3-
phosphate (PI3P) or other phosphoinositide monophosphates, possibly located in lipid rafts. However, these findings still remain controvers ial as evidence to show the existence of PIPs on the exterior membrane remains incomplete, and the mechanisms by which the PIPs may re ach the outer surface of the membrane remain unknown. Importantly, there is still lack of direct cytological evidence showing the translocat ion of effectors through binding of PIPs. Lipd rafts constitute a well-characterized micro-
domain in animal cell plasma membranes that are exploited by many pathogens for host cell entry. Similar small, heterogeneous, highly dy namic, sterol and sphingolipid enriched domains have also been reported in plants, and these might also by exploited by pathogens for macr omolecular trafficking. Based on these considerations, we are investigating the distributions of PIPs, lipid raft proteins, and effectors on the surface of plant cells before and during infection. We have transiently expressed fluorescent protein fusions with a reported plant membran e microdomain protein Remorin with the PI3P-binding protein Vam7P to look at their spatial relationship during plant infection.

643. Controls and Mechanisms of Molecular Biodiversity in Coprophilous fungi. Muhammad Saleem, Muhammad Ishfaq, Muhammad Saleem. Botany, University of the Punjab, New Campus, Lahore, Punjab, Pakistan.

Sordaria fimicola, a model microbe with established genetics had been collected for present study from evolution canyon. The current study reports the development of genetic markers to examine the level of DNA variation in *Sordaria fimicola* in the Evolution Canyon (EC). We developed 20 Exon-primed intron-crossing (EPIC) primer sets from single copy genes based on nucleotide alignments between *S. fimicola* and *Neurospora crassa*. Our initial screen for DNA polymorphisms involved eight field strains of *S. fimicola*, from either slope of the EC. PCR amplification did not detect any nucleotide polymorphism. Two ribosomal protein genes (*RpS29* and *RpS26*) were identified in *S. fimicola* in our initial screen using EPIC primers. Our second approach was to develop simple sequence repeat (SSR) primers based on the available genome sequence of a closely related species, *Sordaria macrospora*. We screened 23 pairs of SSR primers in *S. fimicola*. PCR

amplicons were sequenced and six additional *S. fimicola* specific SSR primers were designed but no genetic variation was observed. *S. fimicola* strains were also assayed biochemically for laccase, superoxide dismutase and catalase enzymes and strains S1, S2, N6 and N7 were found to be most efficient in laccase, superoxide dismutase and catalase production and these strains were further confirmed for their inherited genotypes by amplifying and sequencing the 18S rRNA gene(s). In the last phase of research, laccase, superoxide dismutase and catalase genes were amplified and sequenced. Further various bioinformatics tools such as MAPRes were then used to draw useful conclusions on genetic variation and their effect on picking amino acids (Serine, threonine, and tyrosine) and their role in PTMs and on multifunctionality of isolated/studied enzymes.

644. Screening and Molecular Characterization of Fungi Capable of Laccase Production from Different Forest Ecosystems of Michoacán State, Mexico. Irum Mukhtar. Universidad Michoacana de San Nicolás de Hidalgo M, Lahore, Pakistan.

Utilization of laccases supports to search of new natural sources for laccase enzyme. Previous study also exhibited that various ecosystems have potential to explore laccase producing fungi due to fungal diversity in state of Michoacan in Mexico (Cifuentes *et al.*, 1990; Gomez-Peralta and Gomez -Reyes, 2005). The goal of this study was to explore novel fungal species with laccase activity from different forest ecosystems. Fungal species were isolated from soil and basidiocarp samples collected from five different geographic regions with dominant vegetation of *Pinus* spp., *Abies* spp., and *Quercus spp*. Extracellular laccase activity by fungal isolates were analyzed by guaiacol assay. Identification of laccase positive fungi was carried out by sequence comparison and phylogenetic analysis of internal transcribed region (ITS1F-5.8S-ITS4B) of rDNA gene with reference taxa. A total of thirty (30) laccase producing fungi, representing twenty one (21) species of fifteen (15) genera, were purified. High number of fungal colonies with laccase activity were isolated from basidiocarp and soil samples, collected from Parque Nacional, José Ma. Morelos (Km 23), Charo, Michoacán, Mexico. Among laccase positive isolates, fifteen (15) isolates exhibited strong laccase activity, whereas other showed low laccase indication. Fifteen strains (15) with high laccase activity of basidiomycetes were identified in thirteen genera i.e., *Bjerkandera*, *Corticiaceae*, *Coriolopsis*, *Echinodontium*, *Ganoderma*,, *Hexagonia*, *Irpex*, *Limonomyces*, *Psathyrella*, *Peniophora*, *Phlebiopsis*, *Trametes* and *Trichaptum*. Seven (7) *Trichoderma* and only one *Penicillium* species were found as low laccase producer, isolated from different soil and basidocarp samples. *Trichoderma tomentosum* was recorded as most isolated laccase producing isolate from different soil samples followed by *T. atroviride* from different regions. However, *Penicillium pinophilum* was only laccase producing species of genus *Penicillium*, isolated from soil sample of *Pinus* spp., and *Quercus* spp., dominant area.

645. Structure and function of *Fusarium* communities within the rhizosphere microbiome of two prairie plant species. Nicholas LeBlanc[1], Linda Kinkel[1], H. Corby Kistler[1,2]. 1) Plant Pathology, University of Minnesota, Saint Paul, MN; 2) USDA ARS Cereal Disease Laboratory, Saint Paul, MN.

Fungi in the genus *Fusarium* are well-known producers of secondary metabolites and display high levels of diversity in the rhizosphere where they play functional roles as saprotrophs, plant pathogens, and endophytes. Despite this there is little known about what determines their phylogenetic and functional diversity in soil. Using sequence-dependent methods combined with culture-based phenotyping, the effects of plant community richness and species identity as well as shifts in soil nutrients were investigated for their impacts on *Fusarium* communities in the rhizosphere of two plant species. Soil was sampled from the base of the grass *Andropogon gerardii* and the legume *Lespedeza cpaitata* growing in monoculture and polyculture at the Cedar Creek, Long Term Ecological Research site in Minnesota, USA. Nutrient levels of soil were measured, and soil was used to extract DNAs and to isolate *Fusarium* strains (n=84). Amplicon libraries were created from the extracted soil DNAs targeting a single-copy protein-coding gene and were pyrosequenced. Isolated strains were phenotyped for nutrient use on 95 different carbon substrates, for the presence of genes involved in the production of secondary metabolites using a PCR assay, and for the ability to infect each of the two plant species in the growth chamber. Pyrosequence data showed *Fusarium* community structure was differentiated between monoculture and polyculture plant communities. Richness within different *Fusarium* lineages was influenced by plant community dynamics and soil nutrients. The number of different substrates used by individual cultured *Fusarium* isolates differed between plant species and with soil edaphic levels. Additionally, *Fusarium* isolates testing positive for the presence of particular secondary metabolite genes were non-randomly distributed between plant treatments. Preliminary work suggests that the isolates were not pathogenic, but were able to colonize both plant species. From these data we conclude that plant community dynamics as well soil nutrient levels influence the structure and function of *Fusarium* communities in soil.

646. Occurrence of *Fusarium* and *Microdochium* species and associated mycotoxins in French Wheat. Laetitia Pinson-Gadais[1], Nadia Ponts[1], Emmanuelle Gourdain[2], Guenole Grignon[2], Valerie Laval[3], Helene Batina[3], Christine Ducos[1], Sophie Fromentin[1], Marie-Noelle Verdal-Bonnin[1], Vessela Atanasova-Penichon[1], Christian Barreau[1], Florence Richard-Forget[1]. 1) INRA UR1264 MycSA, 71 avenue E. Bourlaux, CS20032, 33882 Villenave d'Ornon, France; 2) ARVALIS-Institut du végétal Station expérimentale 91720 Boigneville, France; 3) INRA UR 1290 BIOGER - CPP AgroParisTech, France.

In Europe, the contamination of cereals with mycotoxins mainly occur before harvest. Once produced, these mycotoxins can be carried over into feed and food products as a result of their high stability that render decontamination of cereals inefficient. Mycotoxin contamination is therefore of major concern. The toxic and frequently found trichothecenes, especially deoxynivalenol (DON), produced by fungi of the *Fusarium* genus are the most worrying mycotoxins. The issue of wheat contamination by mycotoxins is highly complex for at least two reasons i) different species contaminate simultaneously the wheat grains and ii) these fungi produce concurrently various toxins. This study investigated the relationships between fungal microflora and toxins in kernels, and clarified the impact of agronomic factors on the balance of fungal species. An analytical epidemiological approach was implemented, based on the characterization of 300 wheat samples from 2009-2011 seasons for their contamination by fungal species of the *Fusarium* and *Microdochium* genera, including *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Microdochium majus* and *Microdochium nivale*. Specific qPCR assays were designed to quantify the occurrence of each of the former species in wheat kernels. More than 25000 data were generated analyzed to: i) describe the toxigenic fungal species that can infect French wheat cultivated in various

French regions in relation to various agronomic practices; ii) identify the fungal species that are the major determinant of French wheat contamination with mycotoxins; iii) identify the risk of contamination with fungal species producing emerging toxins.
Such knowledge allows increasing the relevance of risk predictive models for wheat contamination with mycotoxins by integrating a fungal quantitative variable.

647. Antibiotic polymyxin B exhibits novel antifungal activity against *Fusarium* species. Li-Hang Shyu[1], Hsuan-Fu Wang[1], Pei-Lun Sun[2], Ying-Lien Chen[1]. 1) Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan; 2) Department of Dermatology, Chang Gung Memorial Hospital, Linkou, Taiwan.

Fungal plant pathogens cause around 70% of all major crop diseases and dramatically decrease global agriculture production. The genus *Fusarium* comprises several species including *F. oxysporum*, *F. solani*, *F. graminearum* and *F. verticillioides*; of which *F. graminearum* and *F. oxysporum* rank fourth and fifth respectively in a recent survey of top 10 fungal plant pathogens. In clinical settings, *Fusarium*, after *Aspergillus*, is the second most frequent mould to cause invasive fungal infections. *F. solani* and *F. oxysporum* are the first and second prevalent *Fusarium* species to cause clinical diseases. However, there are only few effective antifungal agents available for treating both human and plant *Fusarium* infections. The cationic peptide antibiotic polymyxin B was previously demonstrated to show antifungal activity against the human fungal pathogens *Candida albicans* and *Cryptococcus neoformans*, but its efficacy against *Fusarium* species remained unclear. In current study, we tested the antifungal activity of polymyxin B against twelve *Fusarium* species infecting humans and plants (banana, tomato, melon, pea, wheat and maize). We found that polymyxin B was fungicidal toward all twelve *Fusarium* species, with minimum fungicidal concentrations of 16 or 32 μg/ml for most strains tested, as evidenced by methylene blue staining, spot dilution plating, and XTT reduction assays. Meanwhile, polymyxin B also showed activity against *Pseudomonas aeruginosa*, a bacterial infection frequently associated with *Fusarium* keratitis. Interestingly, in contrast to *C. albicans* and *C. neoformans*, polymyxin B does not show synergistic antifungal activity with medically or agriculturally relevant azoles against *Fusarium* species. In conclusion, our results validate polymyxin B as a potential drug to treat *Fusarium* species infecting both humans and plants.

648. Influence of volatile organic compounds on *Fusarium graminearum* mycotoxin production. Martha Vaughan, Susan McCormick. USDA ARS, Peoria, IL.

Volatile organic compounds (VOCs) are involved in a diverse range of ecological interactions. Due to their low molecular weight, lipophilic nature, and high vapor pressure at ambient temperatures, they can serve as airborne signaling molecules that are capable of mediating inter and intraspecies communications. VOCs emitted by plants can directly contribute to the emitter's disease resistance, enhance the resistance of neighboring plants, and influence sporulation of colonizing fungal species. Correspondingly, fungal VOCs can alter the growth and metabolism of other fungal species and plants. Interestingly, production of a VOC, trichodiene, is the first step in the biosynthesis of Fusarium trichothecene mycotoxins. Despite the close association of this volatile with *in planta* trichothecene contaminants and the function of these mycotoxins as a virulence factor which enhances disease development, not much is known about the potential function of trichodiene as a volatile signal. Governed by the hypothesis that VOCs play a role in the interaction between wheat and *Fusarium graminearum*, the major causal agent of Fusarium head blight disease in wheat, we are investigating the influence of VOCs in mycotoxin production.

649. Diversity of *Fusarium oxysporum* f.sp. *cubence* isolated from local banana cultivars in Indonesia. Nani Maryani[1,2], Fajarudin Ahmad[1,3], Sarah Schmidt[4], Muhammad Ilyas[3], Yuyu Poerba[3], Pedro Crous[5], Gert Kema[1]. 1) Wageningen University and Research Center, Plant Research International, Netherlands; 2) University of Sultan Ageng Tirtayasa (UNTIRTA) Banten, Indonesia; 3) Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia; 4) University of Amsterdam, Netherlands; 5) CBS-KNAW Fungal Biodiversity Center, Utrecht, Netherlands.

Fusarium wilt, known as Panama disease, is one of the major constrains in banana cultivation. The disease is caused by the soil-born fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*) and has devastated banana plantations in almost every banana-growing country. A new strain of *Foc*, commonly known as Tropical Race 4 (TR4), was first detected and disseminated throughout SE Asia since the 1990s and was recently reported in Jordan, Mozambique, Oman, Pakistan and Lebanon. Indonesia is the center of origin for both wild and cultivated banana that likely have co-evolved with *Foc*, hence we hypothesize a wide *Foc* diversity throughout the country. We have recently established a comprehensive collection of 114 *Foc* isolates from the tree main islands of Indonesia: Java, Kalimantan and Sumatra. We isolated *Foc* from the vascular tissue of banana plants showing wilting symptoms. As a preliminary result we morphologically and molecularly characterized – using specific diagnostics - the obtained collection. Around 65% of the isolates belong to TR4. Further extension of the collection and htp-genotyping will enable us to describe the *Foc* landscape in Indonesia.

650. Developing Fungal Resistant Crops at BASF. M. Rodriguez-Carres, M. Wang, D. Waxer, C. Zhao, Y. Han, R. Sasidharan, P. Ren. BASF Plant Science, Research Triangle Park, NC.

Conventional breeding approaches have had limited success in delivering durable fungal resistance and controlling a wide range of fungal pathogens. However, both modern chemical crop protection and plant biotechnology can offer solutions to increase yield and minimize economic losses due to fungal diseases. A major challenge to develop fungal resistant plants is higher resolution and precision of the pathogen-host interface. In order to gain insight into the molecular and cellular dynamics of fungal pathogens in soybean and corn, we have applied state-of-the-art Laser Capture Micro-dissection (LCM) technology coupled with genome-wide RNA sequencing (RNA-seq). LCM-RNAseq increases precision and sensitivity in the detection of gene expression in specific cell types of both fungal and plant cells. Results from these experiments should reveal key molecular processes in response to fungal infection.

651. Identification of a polyketide synthase gene for the synthesis of phleichrome of the phytopathogenic fungus *Cladosporium phlei*. K.-K. So[1], J.-M. Kim[2], S.-M. Park[1], D.-H. Kim[1]. 1) Institute for Molecular Biology adn Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju-si, Jeollabuk-do, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeollabuk-do, South Korea.

Phleichrome, a pigment from a phytopathogenic fungus *Cladosporium phlei*, belongs to a member of fungal perylenequinone. Recently, phleichrome has been intensively studied with regard to its photodynamic activity and potentials to use as a pharmacophore to produce various derivatives for photosensitizers. In order to characterize the biological function of phleichrome and engineer a strain for an enhanced production of phleichrome, we identified a polyketide synthase (PKS) gene, which was responsible for the synthesis of phleichrome. Based on the screening of an ordered gene library of a phleichrome-producing *C. phlei* strain (ATCC 36193), we identified four different PKS genes. Among these four, heterologous expression of a non-reducing PKS gene, *Cppks1*, using *Cryphonectria parasitica* resulted in the production of phleichrome from recombinant *C. parasitica*. These results clearly indicated that the *Cppks1* gene is responsible for the synthesis of phleichrome.

652. Fungal defense against nematodes - Microfluidics to monitor induction of effector proteins in individual hyphae. Stefanie S Schmieder[1], C. Stanley[2], D. van Swaay[2], A. deMello[2], M. Aebi[1], M. Künzler[1]. 1) Institute of Microbiology, ETH Zurich, Zurich Switzerland; 2) Institute of Chemical and Bioengineering, ETH Zurich, Zurich Switzerland.

In the soil biosphere, fungi are confronted with many different antagonists and to combat these, they evolved an impressive arsenal of toxic defense molecules. The production of these effector molecules is often developmentally regulated but can be induced upon confrontation with the antagonist. The regulation, spatial distribution and specificity of this inducible defense response however is poorly understood.

To address these questions, we study the interaction between *Coprinopsis cinerea* and the fungivorous nematode *Aphelenchus avenae. C. cinerea* responds to nematode insult by transcriptional induction of nematotoxic lectins in the vegetative mycelium. To investigate the dynamics of the interaction, we created a *C. cinerea* reporter strain, in which the induction of a toxic effector lectin can be followed by the expression of the dTomato protein. Additionally, we designed microfluidic devices, which allow monitoring of the defense response in individual hyphae, in real time. Using this approach, we showed that the lectin-mediated defense of *C. cinerea* is specific to *A. avenae* and is not triggered by other stimuli, such as bacteria or hyphal damage. By restricting the movement of *A. avenae,* we could further show that *C. cinerea* elicits this defense response only locally and not systemically within the mycelium. However, our data indicate that the mycelium of *C. cinerea* contains different hyphal subtypes of which one is able to transmit this induction over large distances. Finally, the confinement of *A. avenae* within the microfluidic device allowed the extraction of only highly induced mycelial parts for quantitative gene expression analysis. In comparison to conventional methods, which involved analysis of complete mycelial colonies, containing both non-induced and induced hyphae, we found that the level of upregulation for genes implicated in defense, was significantly higher.

653. Cellular interactions between *Coprinopsis cinerea* and bacteria in microfluidic platforms. M. Stöckli[1], C. E. Stanley[2], D. van Swaay[2], P. T. Kallio[1], M. Künzler[1], A. J. deMello[2], M. Aebi[1]. 1) Department of Biology, Swiss Federal Institute of Technology ETH Zurich, Zurich, Switzerland; 2) Department of Chemistry, Swiss Federal Institute of Technology ETH Zurich, Zurich, Switzerland.

The habitat of the model basidiomycete *Coprinopsis cinerea* is the nutrient-rich dung of herbivorous animals that is also populated by various bacteria. Bacteria and fungi share a lifestyle of nutrition by absorption. Due to this trophic competition, strategies to defend the niche have evolved in both organisms but these antagonistic strategies are not well characterized on a cellular level. The visualization of morphological changes of fungal hyphae in co-cultivation experiments is technically challenging due to the three-dimensional growth pattern of a fungal mycelium. To get insight into these interactions new microfluidic platforms were developed. These platforms allow fungal hyphae to grow into microchannels that restrict fungal growth in one dimension and thus reduce the complexity of the fungal mycelium. The platforms are constructed such that bacteria can be added separately and fungi and bacteria can be co-cultivated. This novel technology allowed the observation of a given hyphal compartment in a confined environment and the behavior of a bacterial population over time. We used this platform to investigate the behavior of *C. cinerea* when confronted with *Bacillus subtilis*, a bacterial species known to exhibit antifungal activity. In the presence of *B. subtilis* the hyphae stopped growing after five hours of co-cultivation. The growth stop coincided with the presence of collapsed hyphal compartments and concomitant emergence of membrane-enclosed cytoplasm. Upon confrontation with a *B. subtilis* mutant strain that does not produce the known antifungal lipopeptide, fengycin, *C. cinerea* hyphae did not show collapsed hyphal compartments but were still inhibited in growth. Finally, it was observed that *B. subtilis* cells attached in an end-on manner to only a subset of *C. cinerea* hyphae indicating receptor-mediated interaction and hyphal differentiation within the vegetative mycelium. The difference between these two subsets of hyphae will be analyzed further.

654. Functional groups displayed on substrate surfaces regulated adhesion in various fungi. M. Nishimura[1], M. Nakano[2], K. Miyake[2]. 1) Plant-Microbes Interactions Research Unit, Natl Inst Agrobiol Sciences (NIAS), Tsukuba, Ibaraki, Japan; 2) Surface Interactive Design Group, National Institute of Advanced IndustrialScience and Technology (AIST), Tsukuba,Ibaraki,Japan.

Several plant pathogens, e.g., the blast fungus *Magnaporthe oryzae*, differentiate infection specific structures called appressoria on plastic/glass coverslips as well as on plant surfaces. Together with genetic studies on heterotrimeric G-proteins, it has been considered that recognition signals of surface hydrophobicity are intracellularly transmitted by heterotrimeric G-proteins to trigger appressorium formation in these pathogens. To investigate whether surface hydrophobicity truly contributes to the appressorium formation in *M. oryzae*, we incubated the fungal conidia on substrate surfaces modified with organic molecules by self-assemled monolayer (SAMs) techniques. Our result showed that surface hydrophobicity did not induce appressorium formation; conversely, surface hydroxy group suppressed the fungal differentiation by preventing the fungal attachment to the surface. We further found that hydroxy and oligo(ethyleneglycol) arrayed on substrate surfaces not only prevented adhesion in *M. oryzae* but also weakened/prevented adhesion in major indoor fungi, *Cladosporium*

cladosporoides and *Alternaria alternaria*, filamentous ascomycetes, and *Rhodotorula rubra*, a basidiomycete yeast. Our result showed that certain functional groups displayed on surface suppressed adhesion in a broad range of fungi.

655. Proposal to establish a public DNA repository for fungi. Patrik Inderbitzin, Krishna V. Subbarao. Plant Pathology, UC Davis, Davis, CA.

We propose the establishment of a public DNA repository for the long-term preservation of DNA from all studies in fungal biology. The main goal of the DNA repository is to ensure the continuity of science and the preservation of fungal biodiversity. Storing DNA in a public collection guarantees that identification of species and other taxa can be assessed retroactively. This is particularly useful in the case of ambiguous species boundaries and following taxonomic changes, which can confuse our knowledge of the biology of individual species, and may diminish the value of biological information. The stored DNA is also available for genome sequencing, which in conjunction with existing information tied to individual strains, will significantly advance our understanding of fungal biology. Whereas keeping cultures is the gold standard, storage of DNA requires little maintenance and no government permits, and even large numbers of samples can be stored relatively cheaply. The major disadvantage of DNA over cultures is that DNA cannot be used in biological experiments. However, it may soon be possible to routinely recreate living organism from DNA. A DNA repository could also accept DNA from genomics and metagenomics studies, which would then be available for additional sequencing if needed. To illustrate some of the advantages of a DNA repository, we provide practical examples from the fungal plant pathogen *Verticillium*, the causal agents of Verticillium wilt.

656. Optimizing enzyme cocktails and process conditions for production of cellulosic ethanol. R. Verwaal, H. Pel, M. Hensing. DSM Biotechnology Center, Alexander Fleminglaan 1, 2613 AX, Delft, the Netherlands.

DSM's origin and biotechnology roots date back to 1869 when Jacques C. van Marken, an innovative businessman believing in science, founded NG&SF (Dutch Yeast & Spirits Factory) to produce baker's yeast and potable alcohol.

More than a century (145 years) later, DSM via its joint venture POET-DSM Advanced Biofuels, is again involved in ethanol production, but now via licensing advanced yeast and enzyme technologies to an emerging lignocellulosic bioethanol industry based on corn crop residue. Operation of the joint venture's commercial scale demonstration plant 'Project LIBERTY' in Emmetsburg, Iowa has started up in 2014.

The role of DSM's advanced yeasts and cellulosic enzyme cocktails is to enable the industry to diversify from starch crops to lignocellulosic agricultural residues, unlocking the full industrial potential of this abundantly available, sustainable type of feedstock. While this opportunity has been recognized since many years, enzyme costs were still prohibitive for commercial operations and had to be reduced by a factor of more than 10.

DSM took different approaches to reach this ambitious cost reduction target and developed robust thermostable enzyme cocktails which will a/o be offered as an element of the POET-DSM technology package to parties interested in building their own commercial plants. We will highlight how combined efforts in enzyme discovery and development, fungal strain construction and high throughput fungal strain development, enzyme cocktail optimization, and fermentation as well as application developments were used to make lignocellulosic bioethanol a commercial reality NOW.

657. *rtfA*, a putative RNA-pol II transcription elongation factor gene, is necessary for normal morphological and chemical development, proper response to oxidative stress and pathogenicity in *Aspergillus flavus*. Jessica M. Lohmar[1], Jeffery W. Cary[2], Sourabh Dhingra[1], Ana M. Calvo[1]. 1) Department of Biological Sciences, Northern Illinois University, Dekalb, IL; 2) Agricultural Research Service, USDA, New Orleans, LA.

The filamentous fungus *Aspergillus flavus* is an agriculturally important opportunistic plant pathogen that produces potent carcinogenic compounds called aflatoxins. We identified the *rtfA* gene, ortholog of *rtf1* in *S. cerevisiae* and *rtfA* in *A. nidulans*, in *A. flavus*. Interestingly, *rtfA* has multiple cellular roles in this mycotoxin-producing fungus. In this study we show that *rtfA* regulates conidiation. The *rtfA* deletion mutant presented smaller conidiophores with drastically reduced conidial production compared to the wild-type strain. The absence of *rtfA* also resulted in a strongly decreased or lack of sclerotial production under conditions that allowed abundant production of these resistant structures in the wild type. Additionally, the deletion of *rtfA* notably reduced the amount of aflatoxin B_1 produced, indicating that *rtfA* is a positive regulator of mycotoxin biosynthesis in *A. flavus*. Furthermore, the *rtfA* deletion strain showed a reduction in pathogenicity compared to the wild type on peanut seed as well as in the animal model organism *Galleria mellonella*. A greater sensitivity to oxidative stress, decrease in protease activity and decrease in biofilm formation in the *rtfA* deletion mutant might also contribute to the observed decrease in virulence.

658. High efficiency genome editing in prototrophic and wild strains of *Saccharomyces*. William Alexander[1,2], Drew Doering[1,3], Chris Hittinger[1,2,3]. 1) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 2) DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI; 3) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

Current genome editing techniques available for *Saccharomyces* yeast species rely on auxotrophic markers, limiting their use in wild and industrial strains and species. Taking advantage of the ancient loss of thymidine kinase in the fungal kingdom, we have developed the herpes simplex virus thymidine kinase gene as a selectable and counterselectable marker that forms the core of novel genome engineering tools called the Haploid Engineering and Replacement Protocol (HERP) cassettes. We show that these cassettes allow a researcher to rapidly generate heterogeneous populations of cells with thousands of independent chromosomal allele replacements using mixed PCR

products. We further show that the high efficiency of this approach enables the simultaneous replacement of both alleles in diploid cells. Using these new techniques, many of the most powerful yeast genetic manipulation strategies are now available in wild, industrial, and other prototrophic strains from across the diverse *Saccharomyces* genus.

659. Ecological roles of solanapyrone A produced by plant pathogens *Ascochyta rabiei* and *Alternaria solani*. W. Kim[1], J-.J Park[2], D.R. Gang[2], F.M. Dugan[3], W. Chen[4]. 1) Plant Pathology, Washington State University, Pullman, WA 99164; 2) Institute of Biological Chemistry, Washington State University, Pullman, WA 99164; 3) USDA-ARS Western Regional Plant Introduction Station, Pullman, WA 99164; 4) USDA-ARS Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164.

Ascochyta rabiei and *Alternaria solani*, the causal agents of ascochyta blight of chickpea and early blight of potato, respectively, produce the phytotoxin solanapyrone A. We previously demonstrated that this toxin is dispensable for pathogenicity by comparing virulence of solanapyrone-minus mutants to that of wild-type progenitors. Although the toxin played no role in pathogenicity, the fact that all tested *A. rabiei* isolates produced solanapyrone A prompted us to examine its possible ecological roles in the pathogen's biology. Full genome sequencing of *A. rabiei* revealed that the solanapyrone biosynthesis gene cluster was situated on a subtelomeric region and interspersed with transposable elements. The high similarity of the gene cluster between *A. rabiei* and *Al. solani* and its uniqueness among other related *Ascochyta* species implies the plausibility of horizonal transfer to *A. rabiei* from a distantly related fungal species. Production of solanapyrone A was growth stage-dependent, and its accumulation peaked during asexual fruiting body formation. The expression of key toxin biosynthesis genes was high during saprobic growth on dried chickpea straw, but negligible during plant infection. Concomitantly, solanapyrone A was only detected during the saprobic growth. In agar plate confrontation assays, wild-type strains of *A. rabiei* inhibited saprobic fungi commonly found on chickpea debris, whereas solanapyrone-minus mutants did not. The half maximal inhibitory concentration of solanapyrone A against species in *Alternaria*, *Epicoccum* and *Ulocladium* ranged from 50 to 80 μM. These results suggest that solanapyrone A plays an important role in competition, and presumably in survival of the fungus in nature.

660. Transcriptome and functional analysis of apothecium development in *Botrytis cinerea*. Jan A.L. van Kan[1], Razak B. Terhem[1,2], Alexander Rodenburg[1], Joost H.M. Stassen[1]. 1) Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Department of Forest Management, Universiti Putra Malaysia, 43400 Serdang, Malaysia.

Botrytis cinerea is a plant pathogenic ascomycete producing sexual fruiting bodies named apothecia. We performed RNA-seq analysis of sclerotia, three stages of apothecium development and ascospores. Transcriptomes were compared between successive developmental stages in order to describe transcriptional changes occurring during developmental transitions. Strikingly, more than 5000 genes were differentially expressed between mature apothecia and ascospores. Ascospores expressed several genes encoding virulence factors, even prior to interaction with host plants. Secondly, cluster analysis identified six sets of genes with common transcriptional profiles over the developmental stages. Interestingly, ~80% of the genes that were specifically expressed in mature apothecia were of unknown function and exclusively had homologs within the order Helotiales, but no homologs in fungi producing other types of ascocarps. This suggest that the apothecium is a structure that evolved independently from other fruiting bodies (cleistothecia, perithecia or pseudothecia).

Sexual development in *B. cinerea* is controlled by the *MAT1* locus displaying two alleles, *MAT1-1* and *MAT1-2*, each containing two genes. Besides archetypal genes encoding the alpha-domain protein MAT1-1-1 and HMG-box protein MAT1-2-1, each locus contains one additional gene, designated *MAT1-1-5* and *MAT1-2-4*. Homologs of these genes are only found in closely related taxa, and their function was unknown. Knockout mutants were generated in all four genes in the *B. cinerea MAT1* locus, and crossed with a strain of the opposite mating type, either the wild type or a knockout mutant. Knockout mutants in the *MAT1-1-1* or *MAT1-2-1* gene were entirely sterile. By contrast, mutants in the *MAT1-1-5* and *MAT1-2-4* gene produced stipes, however, these failed to develop an apothecial disc. The *MAT1-1-5* and *MAT1-2-4* mutants show identical phenotypes, suggesting that these genes jointly control the transition from stipe to disk development, probably by acting as transcriptional regulators.

661. Occurrence of a novel mycovirus PoSV and its fungal symptoms in *Pleurotus ostreatus*. H. Choi[1], H. Song[1], D. Kim[2], J. Kim[1]. 1) Bio-Environmental Chemistry Dept, WKU, South Korea; 2) Molecular Biology Dept, JBNU, South Korea.

A novel mycovirus was found in *Pleurotus ostreatus* Halla strain, a bottle cultivated commercial strains of the oyster mushroom in Korea. We attempted to cure the *P. ostreatus* mycovirus (PoSV) in the *P. ostreatus* Halla strain in order to obtain an isogenic virus-free fungal strain as well as virus-infected strain towards contrast. Mycelial fragmentation, followed by being spread on a plate with nylon mesh filtrations resulted in virus-cured strains. Virus curing was verified with gel electrophoresis after dsRNA-specific virus purification and Northern blot analysis using a partial RNA-dependent RNA polymerase-coding gene of PoSV. The growth rate and mycelial dry weight of virus-infected *P. ostreatus* strain were compared to two virus-free isogenic strains with 11 different media. The virus-cured strain was observed higher growth rate than virus-infected strain on culture media czapex-dox, ME, sawdust, V8, and YMG media. In addition, we have explored effects of PoSV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-cured *P. ostreatus* strain was significantly larger than virus-infected *P. ostreatus* strain. These results indicate that the presence of PoSV affect the mycelial growth and fruting body formation of *P. ostreatus*.

662. Curing and functional analysis of the mycovirus (LeV) in *Lentinula edodes* . H. Song[1], J. Kim[1], S. Yun[2], K. So[2], Y. Ko[2], M. Yang[2], D. Kim[2]. 1) Bio-Environmental Chemistry Dept, WKU, South Korea; 2) Molecular Biology Dept, JBNU, South Korea.

This study attempted to cure the edible mushroom *Lentinula edodes* strain FMRI0339 of the *L. edodes* mycovirus (LeV) in order to obtain an isogenic virus-free fungal strain as well as a virus-infected strain for comparison. Mycelial fragmentation, followed by being spread on a plate with serial dilutions resulted in a virus-free colony. Viral absence was confirmed with gel electrophoresis after dsRNA-specific virus purification, Northern blot analysis, and PCR using reverse transcriptase (RT-PCR). Once cured, all of fungal cultures remained virus-free over the next two years. Interestingly, the viral titer of LeV varied depending on the culture condition. The titer from

the plate culture showed at least a 20-fold higher concentration than that grown in the liquid culture. However, the reduced virus titer in the liquid culture was recovered by transferring the mycelia to a plate containing the same medium. In addition, oxygen-depleted culture conditions resulted in a significant decrease of viral concentration, but not to the extent seen in the submerged liquid culture. Although no discernable phenotypic changes in colony morphology were observed, virus-cured strains showed significantly higher growth rates and mycelial mass than virus-infected strains. In addition, we have been explored effects of LeV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-cured strain was significantly larger than virus-infected strain. These results indicate that LeV infection has a deleterious effect on mycelial growth and fruiting body formation.