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## Conifer somatic embryogenesis: from understanding the developmental pathway to the clonal propagation

Caroline Teyssier, Alexandre A. Morel, Jean-François Trontin, Claire C. Le Mette, Philippe Label, Marie-Anne Lelu-Walter

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# 4<sup>th</sup> UPSC-INRA (UPRA) bilateral meeting

26 - 28 March 2012

Umeå Plant Science Centre  
Chemical Biological Centre (KBC)  
Umeå, Sweden

Meeting book with abstracts



## **The Chemical Biological Centre – KBC at Umeå University**

Six departments and two research units at Umeå university (UmU) and the Swedish University of Agricultural Sciences (SLU) are forming one large multidisciplinary research centre: The Chemical Biological Centre (KBC- Kemiskt Biologiskt Centrum). Around 600 people are collaborating in a positive and creative environment in research and teaching. The KBC research school is organizing a graduate program for around 250 PhD students and each department offers a large variety of courses for bachelor and master students.

**[www.kbc.umu.se](http://www.kbc.umu.se)**

*Picture: Mattias Pettersson, Umeå University*

# Welcome to the 4th UPSC-INRA (UPRA) bilateral meeting!

The 4th UPSC-INRA (UPRA) b 26 - 28 March 2012, Umeå Plant Science Centre Chemical Biological Centre (KBC) Umeå University, Sweden

The 2012 bi-annual UPRA meeting will be held in Umeå during the 26th to 28th of March, hosted by the Chemical Biological Centre at Umeå University..

The Umeå Plant Science Center (UPSC, Sweden) and the National Institute for Agricultural Research (INRA, France) have signed a cooperation agreement in 2005 and created a "European Open Laboratory" called UPRA. The dedicated complementarities of both Research Centres in different topics of experimental plant biology and plant genomics has naturally led to the creation of this European open laboratory. One mission of this partnership is to join common efforts on research projects on Plant Biology. A special emphasis is the transfer of knowledge and tools on a model genetic species, *Arabidopsis thaliana*, and the main tree *Populus*. A second mission is to build a European joined structure to take over the training of young scientists through short or long terms exchanges between France and Sweden. The Universities associated to UPRA laboratories are therefore expected to benefit from this privileged cooperation.

This meeting is financed by: UPSC; UPSC Berzelii Centre for Forest Biotechnology; VR; FORMAS, KBC Graduate school and Umeå University.

Umeå University, March 2012

## **The organizing committee**

Catherine Bellini (Catherine.Bellini@plantphys.umu.se) and  
Bjorn Sundberg (Bjorn.Sundberg@slu.se)

<http://www.kbc.umu.se/events/upra-meeting.html>

**Monday March 26**

- 8:30 - 9:00 Registration  
9:00 - 9:15 Welcome / C Bellini - B Sundberg About UPRA  
9:15 - 9:30 Per Gardeström Presentation of the KBC environment  
9:30 - 9:45 Ove Nilsson Presentation of the Umeå Plant Science Center

**Session 1****Chairpersons: Hannele Tuominen / Rishi Bhalerao**

- 9:45 - 10:15 Jean-Denis Faure (AgroParisTech - INRA Versailles)  
*Ins and Outs of Membrane Dynamics, when Acyl Chain Length does Matter*
- 10:15 - 10:45 Stéphanie Robert (UPSC, Dpt of Forest Genetics and Plant Physiology, SLU)  
*A Chemical Genomics Approach to identify genes implicated in vesicular trafficking regulating cell elongation in Arabidopsis thaliana*
- 10:45 - 11:15 Coffee break
- 11:15 - 11:45 Samantha Vernhettes/Hélène Timpano (INRA, Versailles)  
*The Cellulose Synthase Machinery as a target to improve Biomass use*
- 11:45 - 12:15 Totte Niittylä (UPSC, Dpt of Forest Genetics and Plant Physiology, SLU)  
*Carbon partitioning to cellulose in aspen wood*
- 12:15 - 12:45 Edouard Pesquet (UPSC, Dpt Plant Physiology, UmU)  
*Lignification during the life and death of xylem vessels*
- 12:45 - 14:00 Lunch
- 14:00 - 16:00 Poster session
- 16:00 - 16:30 Coffee break

**Session 2****Chairpersons: Gunnar Wingsle / Thomas Moritz**

- 16:30 - 17:00 Véronique Santoni (INRA/CNRS/SupAgro - Montpellier)  
*Regulation of root aquaporins in response to abiotic stimuli - a proteomic approach*
- 17:00 - 17:30 Marie-Béatrice Bogeat-Triboulot (INRA/Univ. Lorraine - Nancy)  
*Aquaporins TIP1s expression and their regulation in the growing root apex of poplar under osmotic stress*
- 17:30 - 18:00 Stefan Björklund (Medical Biochemistry and Biophysics, Umeå Univ.)  
*The Arabidopsis mediator, Function in plant development, Stress response and light signaling*
- 18:00 - 18:30 Johannes Hanson (UPSC, Dpt Plant Physiology, UmU)  
*Dynamic protein composition of Arabidopsis thaliana cytosolic ribosomes in response to sucrose feeding, as revealed by quantitative proteomics*

**18:30 Diner on your own**

**Tuesday March 27****Session 3****Chairpersons: Karin Ljung / Markus Grebe**

- 8:30 - 9:00 Teva Vernoux (CNRS/INRA/ENS Lyon)  
*Hormone signaling integration during dynamic morphogenesis at the shoot apex*
- 9:00 - 9:30 Catherine Rameau (INRA Versailles)  
*PsBRC1, the pea homolog of the maize TB1 gene is an integrator of strigolactone and cytokinin signalling pathways for the control of shoot branching*
- 9:30 - 10:00 Urs Fischer (UPSC - Dpt of Forest Genetics and Plant physiology, SLU)  
*STM and KNAT1 are required for the terminal differentiation of xylem fibers in the Arabidopsis hypocotyl*
- 10:00 - 10:30 Caroline Teyssier (INRA - Orléans)  
*Conifer somatic embryogenesis: from understanding the developmental pathway to the clonal propagation*
- 10:30 - 11:00 Coffee break
- 11:00 - 11:30 Edward Businge (UPSC - Dpt of Forest Genetics and Plant physiology, SLU)  
*Regulation of somatic embryogenesis in Norway spruce: a metabolomics approach*
- 11:30 - 12:00 Philippe Nacry (INRA/CNRS/SupAgro - Montpellier)  
*The Arabidopsis NRT1.1 transporter acts as a nitrate sensor and governs root colonization via modification of local auxin concentration*
- 12:00 - 12:30 Valérie Legué (INRA/Univ. Lorraine - Nancy)  
*Regulation of adventitious root and lateral root development in poplar*
- 12:30-13:00 Laszlo Bakó (UPSC - Dpt of Plant Physiology, UmU)  
*Differential responses of the RBR-E2F pathway to auxin in the Arabidopsis root*
- 13:00 - 14:00 Lunch  
Visit of UPSC / KBC facilities (Microscopy, Metabolomics/Proteomics, Growth facilities, Somatic Embryogenesis...platforms)  
Informal discussion  
Site seeing
- 19:00 Conference dinner

**Wednesday March 28****Session 4****Chairpersons:** Göran Samuelsson / Vaughan Hurry

- 9:00 - 9:30 Catherine Campbell (UPSC - Dpt of Plant Genetics and Plant Physiology, UmU)  
*Potential signals for shifts in carbon allocation in response to nitrogen fertilisation*
- 9:30 - 10:00 Benjamin Petre (INRA/Univ. Lorraine - Nancy)  
*Poplar immunity at stake: new insight into the search of leaf rust effectors*
- 10:00 - 10:30 Benedicte Albrechtsen (UPSC - Dpt of Plant Physiology, UmU)  
*Defense chemicals in European aspen and associated arthropod community structure*
- 10:30 - 11:00 Coffee break
- 11:00 - 11:30 Frédéric Gaymard / Marc Bournier (INRA/CNRS/SupAgro-Montpellier)  
*When iron meets light: regulation of iron homeostasis by light, circadian clock and oxidative stress.*
- 11:30 - 12:00 Nicolas Rouhier (INRA/Univ. Lorraine - Nancy)  
*Plastidial glutaredoxins: glutathione-dependent enzymes involved in detoxification, redox signalling and iron-sulfur cluster biogenesis*
- 12:00 - 12:30 Anita Sellsted (UPSC - Dpt of Plant Physiology, UmU)  
*Ethanol production from lignocellulose using a filamentous biocatalyst*
- 12:30 - 13:00 Lunch
- 13:00 - 15:00 Poster session
- 15:00 - 15:30 Coffee break

**Session 5****Chairs:** Stefan Jansson / Maria Ericksson

- 15:30 - 16:00 Torgeir Hvidsten (UPSC - Dpt of Plant Physiology, UmU)  
*Complexity and conservation of regulatory networks in plants*
- 16:00 - 16:30 François Parcy (INRA/CNRS/CEA/Grenoble Univ. - Grenoble)  
***Function and evolution of the LEAFY transcription factor***
- 16:30 - 17:00 Françoise Monéger (CNRS/INRA/ENS Lyon)  
*A data-driven integrative model of sepal primordium polarity in Arabidopsis*
- 17:00 - 17:30 Nathaniel Street (UPSC - Dpt of Plant Physiology, UmU)  
*Sequencing and assembly of the Norway spruce (Picea abies) genome*
- 17:30 - 17:45 Concluding remarks - decision about next meeting
- 19:00 Diner at Sävargården (Invited speakers, chairpersons and organizers)

# *Talks*



## Ins and outs of membranodynamics, when acyl chain length does matter

Jean-Denis Faure

Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, INRA Centre de Versailles-Grignon, Route de St. Cyr (RD10), 78026 Versailles Cedex France

Very long chain fatty acids (VLCFAs) are fatty acids with an acyl chain of 18 carbons and longer. They are elongated by the elongase complex in the endoplasmic reticulum and are incorporated into four major lipid pools (triacylglycerols, waxes, phospholipids, complex sphingolipids) (1). Functional analysis of several components of the elongase complex demonstrated the essential role of VLCFAs in plants, invertebrates and vertebrates. Identification of the acetyl-CoA carboxylase PASTICCINO3 and the 3-hydroxy acyl-CoA dehydratase PASTICCINO2 revealed that VLCFAs are important for cell proliferation and tissue patterning (2, 3) but also for cytokinesis (4). VLCFA elongation also required the ER-localized immunophilin PASTICCINO1 (PAS1) and that PAS1 impairment resulted in defective polar auxin transport and tissue patterning during plant development (5). Interestingly, very long acyl chains are major components of sphingolipids that are essential for vesicular trafficking and cell polarity in yeast and mammals. A key step of sphingolipid biosynthesis is the acylation of long chain bases catalyzed by the sphingoid base N-acyl transferase or Ceramide synthase. Genetic and pharmacological analysis of VLCFA-ceramide synthase activity revealed several developmental defects related to defective polar auxin transport. These defects were associated with specific modification of subcellular trafficking and membrane dynamics (6). The specific role of acyl chain length of membrane lipids in vesicular trafficking and cell polarity during plant development will be discussed.

1. Bach L & Faure JD (2010) C R Biol 333(4):361-370.
2. Bach L, et al. (2008) Proc. Natl. Acad. USA 105(38):14727-14731.
3. Baud S, et al. (2004) EMBO Rep 5(5):1-6.
4. Bach L, et al. (2011) Journal of cell science 124(Pt 19):3223-3234.
5. Roudier F, et al. (2010) Plant Cell 22(2):364-375.
6. Markham JE, et al. (2011) Plant Cell 23(6):2362-2378

## **A chemical genomic approach to identify genes implicated in vesicular trafficking regulating cell elongation in *Arabidopsis thaliana***

**Stephanie Robert**

SLU/Umeå Plant Science Center, Department of Forest Genetics and Plant Physiology, 901 83 Umeå SWEDEN

The chemical genomics approach uses small molecules to modify or disrupt the function of specific proteins. It provides a novel avenue for rapid and effective dissection of biological mechanisms and gene networks in ways not feasible with mutation-based approaches. Vesicular trafficking is an essential cellular process driving the distribution of cargos within cells and maintaining subcellular structure. It basically underlies all cellular functions and can be modulated by both developmental and environmental signals. Moreover, cell wall plays a central role in plant development such as the regulation and control of growth anisotropy and cell shape. Understanding cellulose synthesis and deposition is therefore essential for understanding plant growth, development and evolution. The biosynthesis and deposition of cell wall components rely on vesicular trafficking, which involves vesicle formation, transport and fusion with target membranes. Despite the tremendous importance of this process, our knowledge on the underlying mechanism and the regulatory networks is very limited. In this study, we have developed a pollen-based high-throughput screen to select chemicals as inhibitors of pollen germination, a process absolutely dependent on intact vesicular trafficking. Using cellulose biosynthesis enzyme GFP-tagged, on a secondary screen, we are dissecting a network of genes controlling vesicle trafficking involved in cell wall biosynthesis. By this approach, we expect to obtain novel regulators of cellulose biosynthesis, which will be instrumental to further dissect mechanism of plant development.

## The cellulose synthase machinery as a target to improve biomass use

Hélène Timpano, Olivier Darracq, Eric Badel, Volker Bischoff, Thierry Desprez, Brigitte Pollet, Catherine Lapierre, Sylvie Citerne, Richard Sibout, Herman Höfte, Samantha Vernhettes and Martine Gonneau.

IJPB-UMR1318, INRA, Route de St Cyr, 78026 Versailles, France, [www-ijpb.versailles.inra.fr/](http://www-ijpb.versailles.inra.fr/)

The production of second-generation biofuels based on the transformation of plant biomass is a pressing issue. Biomass is represented by cell walls of the plant cells consisting of a network of cellulose microfibrils and polysaccharides encrusted by lignin. To enhance the potential of plant biomass, we need to provide insights on the mechanisms of the biosynthesis of cell wall polymers. For example, it is important to improve the saccharification yield of cellulose microfibrils to produce the highest amount of bioethanol. We therefore combine studies on the well-known model plant *Arabidopsis* and *Brachypodium distachyon*, the new model species for temperate gramineae and monocotyledonous crops dedicated to biofuel production.

Cellulose is synthesized by plasma membrane-bound cellulose synthase complexes (CSC) containing cellulose synthase proteins (CESAs) and requires other partners among which the endo-beta1,4 glucanase KOR1. The intracellular trafficking of CESAs seems to be crucial to regulate the cellulose synthesis rate. We are now investigating in detail the intracellular trafficking of KOR1 and the impact of KOR1 phosphorylation on its localization in *Arabidopsis* dark-grown hypocotyls.

In parallel we selected by visual screening of the Versailles collection of mutagenized *Brachypodium distachyon* a mutant called spa. This mutant shares characteristics of the brittle culm mutants of rice and barley, such as brittleness, irregular xylem, and a cellulose content deficiency especially in stems, with 50% of the amount found in the wild type. Lignin assays indicate a higher amount of lignin in spa. Interestingly, this mutant is also "floppy" unlike others brittle culm mutants which are fully erected and the mechanical strength defects of spa is illustrated by a Young's modulus three times lower than that of WT. Complementary approaches are in progress to identify the SPA gene: sequencing of candidate genes related to cell wall synthesis or co-expressed with secondary cell wall cellulose synthases and a classical mapping strategy combined with NGS methods. Moreover within the framework of the European RENEWALL and KBBE CellWall projects and thanks to the co-expression network tool BradiNet (M. Mutwill, KBBE project), RNAi strategies are in progress to inactivate a few genes selected according to specific expression criteria and potentially involved in cell wall synthesis specifically in monocots. Among these genes we are focusing on the MAP65 family (Microtubules Associated Proteins), which could play a role in cellulose deposition according to the close relationship between microfibrils and microtubules.

## Carbon partitioning to cellulose in aspen wood

Melissa Roach<sup>a</sup>, Lorenz Gerber<sup>a</sup>, Bo Chang<sup>c</sup>, András Gorzsás<sup>a</sup>, Mattias Hedenström<sup>b</sup>, Ingo Burgert<sup>c</sup> Björn Sundberg<sup>a</sup> and **Totte Niittylä<sup>a</sup>**

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<sup>c</sup>ETH Zurich, Institute for Building Materials, Zurich, Switzerland. [www.ifb.ethz.ch/](http://www.ifb.ethz.ch/)

Sucrose is the main transported form of carbon in several plant species including the model tree aspen. Sucrose metabolism in developing wood is therefore central for the regulation of carbon partitioning to stem biomass. In order to be integrated into metabolism sucrose must be cleaved by either sucrose synthase (SuSy) or invertase. Cleavage with SuSy produces UDP-glucose and fructose, whereas invertase produces glucose and fructose. SuSy has previously been hypothesized to channel UDP-glucose into cellulose biosynthesis. To investigate the role of SuSy in wood formation we characterized transgenic lines of aspen with almost absent SuSy activity in developing wood. The analyses revealed a decrease in all wall polymers and a dramatically altered cell wall ultrastructure pointing to an important role for SuSy in wood cell wall biosynthesis. However, cellulose biosynthesis was not abolished and no strong growth phenotypes were observed. This suggested that just like in *Arabidopsis* SuSy is not essential for cellulose biosynthesis. Regardless whether SuSy or invertase cleaves sucrose, half of the sucrose-derived carbon is fructose. RNAi mediated reduction of fructokinase (FRK2) activity in developing wood led to accumulation of soluble neutral sugars and a decrease in hexose phosphates and UDP-glucose indicating that carbon flux to the cell wall polysaccharide precursors was decreased. Reduced FRK2 activity also led to thinner fiber cell walls with a reduction in the proportion of cellulose. No pleiotropic effects on stem height or diameter growth were observed. These results established a central role for the FRK2 activity in carbon flux to wood cellulose.

## Lignification during the life and death of xylem vessels

**Edouard Pesquet**

Umeå Plant Science Centre (UPSC), Department of Plant Physiology, Umeå University, Umeå, S-90 187, Sweden

Lignification and programmed cell death (PCD) are of fundamental importance to the production of functional tracheary elements (TEs) – a cellular corpse which requires PCD to hollow out its content and lignification to reinforce its wall for efficient raw sap [1]. Live cell imaging of TE formation suggested that lignification occurred after TE PCD [2]. The interplay between PCD and lignification during TE formation was studied at the cellular level by pharmacological means in the *in vitro* differentiating cell cultures, at the genomic level using suppression subtractive hybridization (SSH) libraries and at the whole plant level by analyzing the cell wall compositions of 51 *Arabidopsis* KO mutants homologous to the identified SSH clones. Ethylene, previously shown to be associated with TE formation [3], was pharmacologically altered in the differentiating TEs and blocked both lignification and PCD without affecting secondary cellulose formation. Pharmacological modulation of TE lignin monomer biosynthesis resulted in dead unlignified TEs, but which could partially relignify when supplied with extracellular lignin monomers. SSH libraries constructed from TE differentiating cell cultures treated with or without STS allowed to identify 693 differentially expressed genes involved in PCD-triggered lignification. Among these genes were identified known cell wall biosynthesis, lignin monomer biosynthesis and PCD related genes. Interestingly, cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD), two lignin monomer synthesis genes affected by STS, were expressed beyond normal TE lifespan. *In situ* localization using IS-RT-PCR of CAD and CCR revealed that both genes were expressed in cells analogous to xylem parenchyma. Cell wall composition analysis of 51 *Arabidopsis thaliana* mutants homologous to SSH candidate genes were characterized by reverse genetic approaches and confirmed that lignin monomeric composition was affected in the whole plant context [4]. Altogether, our results suggest that lignin is mostly made through a post-mortem and cooperative process in xylem vessels.

### References:

- [1] Menard D. and Pesquet E. (2012) Wood in a tube: development of *in vitro* wood cell differentiating systems. *Journal of Experimental Botany*, in press.
- [2] Pesquet E., Korolev AV, Calder G. and Lloyd C.W. (2010) The microtubule-associated protein AtMAP70-5 regulates secondary wall patterning in *Arabidopsis* wood cells. *Current Biology*, 20, 744-749.
- [3] Pesquet E., and Tuominen H. (2011) Ethylene stimulates tracheary element differentiation in *Zinnia* cell cultures. *New Phytologist*, 190, 138-149.
- [4] Pesquet E., Zhang B., Puhakainen T., Barbier O., Gerber L., Escamez S., Gorzas A., Courtois-Moreau C., Alatalo E., Paulin L., Kangasjarvi J., Sundberg B., Goffner D. and Tuominen H. (2012) Non-cell autonomous lignification of tracheary elements, *submitted*.

## Regulation of root aquaporins in response to abiotic stimuli – a proteomic approach

Magali di Pietro\*, Jérôme Vialaret<sup>†</sup>, GuoWei Li\*, Michel Rossignol<sup>†</sup>, Christophe Maurel\*, **Véronique Santoni\***

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Aquaporins are water channels that facilitate the transport of water across plant cell membranes and play a critical role in the regulation of plant water status in response to changing environments. We used a proteomic approach to address the mechanisms involved in the response of root water permeability in *Arabidopsis* to 7 representative stimuli including water and ionic stresses, nutrient availability, sucrose, reactive oxygen and nitrogen species, and a bacterial elicitor.

The effects of stimuli on the root hydraulic conductivity (L<sub>pr</sub>) were described with emphasis on kinetic responses. All treatments induced a decrease in L<sub>pr</sub>, within minutes (hydrogen peroxide, nitric oxide), hours (salt, mannitol) or days (nitrate or phosphate deprivation; prolonged night i.e. sucrose starvation). Whereas a resupply of nitrate did not change L<sub>pr</sub>, phosphate or sucrose resupply induced a partial recovery of L<sub>pr</sub>.

A single procedure allowing the combined fractionation of non-modified peptides and the purification of phosphopeptides was used by combining off-line Strong Cation eXchange and Titanium Dioxide chromatographies. For the quantitative approach, large scale semi-quantitative proteomics and phospho-proteomics was performed by label-free comparison of liquid chromatography (LC)-MS profiles (nanoLC-QTOF). This integrated workflow allowed the identification of 1383 proteins (21 out of 35 aquaporins), including 389 phosphoproteins with 706 phosphosites of which 66% were novel (16 aquaporin phosphosites of which 7 were novel). Extensive analysis of aquaporin proteomic data revealed that, over the 7 physiological contexts investigated, L<sub>pr</sub> is not correlated to the total abundance of aquaporins. The data rather suggest that environmental regulation of L<sub>pr</sub> results from multifactorial mechanisms including the phosphorylation of the C-terminal part of PIP<sub>2</sub>;1/2;2/2;3 and of the N-terminal part of PIP<sub>1</sub>;1/1;2. In the case of tonoplast aquaporin TIP<sub>2</sub>;3, a dramatic post-translational regulation in response to salt stress and a role in root hydraulics were uncovered.

## **Aquaporin TIP1s expression and their regulation in the growing root apex of poplar under osmotic stress**

Rémy Merret<sup>1</sup>, Irène Hummel<sup>1</sup>, Bruno Mouliat<sup>2</sup>, David Cohen<sup>1</sup> and **Marie-Béatrice Bogeat-Triboulot<sup>1</sup>**

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<sup>2</sup>INRA, Université Blaise Pascal, UMR\_A547 Physique et Physiologie Intégratives de l'Arbre Fruitier et Forestier, Domaine de Crouelle, F-63039 Clermont Ferrand, France

Aquaporins form a superfamily of intrinsic channel proteins that facilitate the bidirectional transfer of water and other small neutral molecules across biological membranes (Maurel et al, 2008). The past two decades revealed that these proteins play an important role in plant water balance, notably in the response to water deficit. At the cell scale, some aquaporins were shown to be involved in the control of the water flow required for cell expansion (Chaumont, 1998; Hukin, 2002, Volkov, 2007). This study focuses on the expression of the TIP1 aquaporin subfamily, in the growing root apex of poplar cuttings grown in hydroponics and submitted to two levels of osmotic stress. A conceptual framework, combining growth kinematics and transcript density at a high spatial resolution in a fluid mechanics formalism, was used to describe the regulation of gene expression in time and space along the root apex (Merret et al, 2010). The 8 TIP1s showed specific expression patterns along the root apex. Under low osmotic stress, for which growth rate was restored to the control level, spatial expression patterns were almost not affected. Under strong osmotic stress, the growth rate reduction was associated with a shift of maximal transcript densities towards the tip but the relative expression level was not affected or slightly reduced. PtTIP1;4 density pattern overlapped the REGR profile, whatever the conditions. Moreover, the regulation of its expression had a similar temporal pattern in all treatments, even if the size of the growth zone varied, supporting the hypothesis of a functional link between TIP1;4 and cell expansion. We show that the conclusions derived from the analyses of these dynamic processes, cell expansion and gene expression, are influenced by the way time and space are considered.

## The *Arabidopsis thaliana* Mediator; function in plant development, stress response and light signalling

Stefan Björklund

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Mediator is a multisubunit protein complex which functions as a co-regulatory transcription factor by conveying signals from promoter-bound transcription factors (activators and repressors) to the general RNA polymerase II (pol II) transcription machinery. Mediator can be divided in three subdomains called head, middle and tail where tail is believed to make contact with activators and repressors and head with pol II. As sessile organisms, plants need to respond to abiotic signals such as day length, light quality, temperature, drought, and salinity. These signals are sensed by different receptors and transmitted by signal transduction pathways to finally result in a proper gene expression response. Mediator is one of the final points for integration of these signals but the molecular mechanisms for such integration and for their transmission to the general transcription machinery have remained elusive. We recently identified three transcription factors which are involved in different stress response pathways and showed that they all interact with the same domain of the Med25 mediator subunit. We also find that the interaction between one of the transcription factors, Dreb2A and Med25 is involved in repression of PhyB-mediated light signalling. Mediator thus integrates signals from different regulatory pathways. We will present results from a large scale study of developmental, phenotypic and metabolic effects caused by inactivation of a majority of the Mediator subunits.  This work was supported by grants from the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning, the Swedish Cancer Society, the Swedish Research Council, the Swedish Governmental Agency for Innovation Systems, and the Kempe Foundation.



## **Dynamic protein composition of *Arabidopsis thaliana* cytosolic ribosomes in response to sucrose feeding, as revealed by quantitative proteomics**

Maureen Hummel and Johannes Hanson

Molecular Plant Physiology, Utrecht University, The Netherlands and Umeå Plant Science Center, Umeå University, Sweden.

Ribosome biogenesis and mRNA translation are very energy requiring cellular processes. *Arabidopsis thaliana* ribosomes consist of seventy-nine different ribosomal proteins that each are encoded by two to six (paralogous) genes. Previous reports have shown that the vast majority of these r-protein paralogs are expressed at both the mRNA and protein level. However, it is unknown whether all ribosomal protein paralogs are incorporated into the ribosome and whether the relative incorporation of r-protein paralogs varies in response to environmental cues. We have approached this directly by quantitative proteomic analysis of immunoprecipitated *A. thaliana* cytosolic ribosomes. Our results show that the vast majority of ribosomal protein paralogs are present in cytosolic ribosomes. In addition, we show that the protein composition of cytosolic ribosomes changes in response to manipulation of the metabolic status by sucrose feeding. The implications of these findings on the regulation of translation will be discussed.

## **Hormone signaling integration during dynamic morphogenesis at the shoot apex**

**Teva Vernoux**

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Plant organs are initiated at the shoot apex in precise spatio-temporal patterns that are the primary determinants of phyllotaxis, the geometric arrangement of organs along the stem. Self-organizing local accumulation of the hormone auxin and inhibitory fields produced by depletion around organs through active transport have been proposed to be sufficient to generate the dynamics of these patterns. We have performed a systems biology analysis of the auxin signaling pathway combining large scale-analysis of the expression and interactions between the effectors of the pathway, the development of a new auxin signaling sensor and mathematical modeling. We have also analyzed the function of a negative regulator of cytokinin signaling, the Arabidopsis Phosphotransfer Protein 6 (AHP6), in the dynamics of organ initiation. Our results highlight a key role for hormone signaling pathways alongside the auxin transport system in providing robustness to the spatio-temporal dynamics of organogenesis at the shoot apex.


## **PsBRC1, the pea homolog of the maize TEOSINTE BRANCHED1 (TB1) gene is an integrator of strigolactone and cytokinin signalling pathways for the control of shoot branching**

De Saint Germain Alexandre<sup>1</sup>, Pillot Jean-Paul<sup>1</sup>, Braun Nils<sup>1</sup>, Boyer François-Didier<sup>2</sup>, **Rameau Catherine<sup>1</sup>**

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Strigolactones (SLs) are small carotenoid derived molecules known to be produced by plant roots and released into the rhizosphere to play the same role of signalling the proximity of a host root in both symbiotic and parasitic interactions. They have been recently recognised as a novel plant hormone controlling shoot branching in seed plants (Gomez-Roldan et al., 2008; Umehara et al., 2008). The identification and characterisation of high branching mutants in pea, Arabidopsis and rice demonstrated that these mutants were either SL deficient or not responding to SL applications. Other SL functions in plant development have been recently demonstrated such as cambium activity stimulation (Agusti et al. 2011) or plant height control (unpublished data).

We are currently investigating the functions of two novel genes involved in SL response, to integrate them into the pea model of the control of branching: on one hand PsBRC1, the pea homolog of the maize TEOSINTE BRANCHED1 (TB1), and on another hand RMS3, the homolog of the rice D14 gene encoding a protein of the  /hydrolase superfamily (Arite et al. 2009). GID1, the gibberellin (GA) receptor is also member of this family (see Poster of A. De Saint Germain).

PsBRC1 encodes a transcription factor of the plant specific TCP family, named from the first three identified members, TB1, CYCLOIDEA (CYC) from *Antirrhinum majus* and PCF-coding genes from rice (Braun et al., 2011). We propose that PsBRC1, almost exclusively expressed in the axillary bud, may provide the link between systemic signalling (cytokinin, SL) and events occurring within the axillary bud to control bud outgrowth (Braun et al., 2011; Dun et al., 2011). A few examples of the use of PsBRC1 as a molecular marker for SL signalling will be given e.g. in Structure-Activity Relationships (SAR) studies, to gain a better understanding of the difference of activities shown by different SL-related molecules in the control of branching.

## STM and KNAT1 are required for terminal differentiation of xylem fibers in the *Arabidopsis* hypocotyl

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Secondary xylem in *Arabidopsis* hypocotyls is characterized by two developmental phases; firstly cambial derivatives differentiate into xylem vessels and parenchymatic cells, and secondly, upon flowering in place of parenchymatic cells, differentiation into xylem fibers with lignified secondary cell walls take place. Weak *stm* and *knat1* mutants were impaired in the terminal differentiation of xylem fibers of the hypocotyl, while cambial activity and flowering time were not affected. In a *stm;knat1* double mutant fiber differentiation was completely blocked while residual cambial activity could still be observed. Interestingly, expression of *SND1* and *NST1*, as well as their downstream targets involved in secondary cell wall synthesis were almost abolished in the mutants. Furthermore, the expression of *GA20* and *GA3* oxidases were strongly reduced in the *stm;knat1* double mutant. Differentiation of xylem fibers could be partially restored in *stm* by the application of gibberellin.

De-repression of *KNOX* gene expression in *bop1;bop2* correlated with an increased degree of secondary xylem differentiation, while over-expression of *BOP2* phenocopied weak *stm* and *knat1* mutants. This latter finding indicates that the degree of tissue differentiation in the secondary xylem of the hypocotyl depends on *STM* and *KNAT1* activity in a quantitative manner.

As opposed to their function as anti-differentiation signals during primary growth in the shoot apical meristem, *KNAT1* and *STM* positively regulate the differentiation of cambial derivatives in the hypocotyl.

## **Conifer somatic embryogenesis: from understanding the developmental pathway to clonal propagation**

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Advances in plant biotechnology offer new opportunities, in the field of plant propagation and genetic engineering. Development of clonal propagation method, such as somatic embryogenesis, has potentially numerous applications. Indeed, this efficient method of plant regeneration constitutes a tool for research (study of gene function) and for species improvement (production of a large number of genetically improved plants). The amenability of embryogenic cultures to storage at very low temperature (-196°C) enables long-term preservation of cryogenic collections of improved resources in a juvenile state. So somatic embryogenesis is the key enabling technology, for genetic engineering and molecular marker development for marker-aided selection of elite trees. This technology is also providing a basic research tool for genomics researches such as proteomic and transcriptomic studies.

Since the 90's, INRA engaged researches on somatic embryogenesis in *Pinus pinaster* that remains difficult in particular somatic embryo maturation. In order to control their quality physiological and molecular markers have been developed and compared with zygotic embryos (control). Approaches such as transcriptomic and proteomic will be presented.

## Regulation of embryo development in conifers: A metabolomics approach to investigate the basic mechanisms of embryo development

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Norway spruce (*Picea abies*) is an economically significant tree species native to northern and central Europe. Somatic embryogenesis (SE) presents an in vitro strategy for clonal propagation of tree species that are difficult to propagate vegetatively using cuttings. Deployment of the SE process for industrial scale production of Norway spruce plants is however hindered by embryogenic cell lines with different degrees of success in embryo development. To better understand the differences in embryo development among cell lines, we analyzed the metabolic profiles of cell lines with normal, aberrant and blocked embryo development. Using manual time lapse photography we followed embryo development from pro embryogenic masses (PEMs) to mature embryos in each of the three cell line. Metabolite profiles for each cell line during the course of somatic embryo development were compiled using Gas Chromatography coupled with Mass Spectrometry (GC/MS). Key metabolites were identified for each developmental stage in all cell lines using Multivariate statistical analysis tools. Potential candidate metabolites significant for normal embryo development were identified.

## **The *Arabidopsis* NRT1.1 transporter acts as a nitrate sensor and governs root colonization via modification of local auxin concentration**

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Nitrate ( $\text{NO}_3^-$ ) is both the main nitrogen source for nutrition of higher plants, and a signal molecule regulating their metabolism and development. The roots sense the  $\text{NO}_3^-$  concentration in the soil solution, and trigger signalling pathways allowing plant adaptation to changes in the external availability of this key nutrient.

Localized proliferation of lateral roots in  $\text{NO}_3^-$ -rich patches is a striking example of the nutrient-induced plasticity of root development. On the basis of a fine analysis of root development, we showed that mutants of NRT1.1 displayed a strongly decreased root colonization of  $\text{NO}_3^-$ -rich patches, resulting from reduced lateral root elongation and delayed lateral root emergence. We then concluded that NRT1.1 acts as a  $\text{NO}_3^-$ -sensor and regulates the expression of a MADS box transcription factor, ANR1, necessary for colonization of  $\text{NO}_3^-$  rich patches (Zhang and Forde 1998) and enables the plant to detect local  $\text{NO}_3^-$  concentration and govern local root growth.

Further characterization of the underlying signalling mechanism showed that NRT1.1 acts not only as a  $\text{NO}_3^-$  transporter but also facilitates influx of the phytohormone auxin in a nitrate concentration dependant manner. When external  $\text{NO}_3^-$  concentration is low, NRT1.1 transports auxin out of the lateral root primordium preventing its accumulation and thus lateral root development. This defines a new mechanism for sensing environmental stimuli, and for connecting nutrient and hormone signalling in the control of organ development.

Besides, NRT1.1 was shown to regulate the expression of hundreds of genes (“primary  $\text{NO}_3^-$  response genes”) in response to  $\text{NO}_3^-$  supply and recently Ho et al (2009) delineated the signalling pathway involved and identified point mutations in the NRT1.1 protein that alter the  $\text{NO}_3^-$  transport activity but not the signalling function. The involvement of this signalling pathway and the effect of point mutations on the nitrate dependant root growth will be discussed.

## Regulation of adventitious and lateral root development in poplar

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The ability to rapidly form numerous adventitious roots provides a selective advantage for plant species that vegetatively propagate. The genus *Populus* is a typical example of woody species that are propagated by direct planting of stem cuttings in the field. Despite the importance of adventitious rooting, the mechanism underlying this developmental process remains poorly understood.

Using genomic approaches, we identified the transcriptional profiles associated with the different developmental stages of adventitious root formation in the model tree species *Populus*. Analysis of the gene expression data indicates and genetic tools show that the PtAIL1 (AINTEGUMENTA LIKE 1) transcription factor of the AP2 family is a key regulator acting early in the regulation of adventitious rooting.

Microarrays data revealed that some genes involved in auxin pathways are regulated during the formation of adventitious root. We are investigating the potential function of these genes in the frame of UPSC/INRA cooperation (I. Perrone's poster). Since easy-to-root and difficult-to-root genotypes are well known in the genus *Populus*, we want to determine the putative role of these genes by comparing their expression in two different species *P. trichocarpa* (easy-to-root) and *P. tremula* (difficult-to-root) during the adventitious root initiation in stem cuttings.

The root system architecture in poplar includes lateral root formation, which are initiated from adventitious roots. In another recent study we showed that auxin signaling is implicated in the alteration of the root system architecture in response to fungal signals. We identified PtaPIN transcripts such as PtaPIN9, an orthologue of AtPIN2, involved in auxin efflux transport, that are modulated during the interaction (A. Vayssières's poster).



## **Differential responses of the RBR-E2F pathway to auxin in the *Arabidopsis* root**

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The Retinoblastoma-related protein (RBR) of *Arabidopsis* is a critical regulator of cell division, differentiation and development. To dissect the role of RBR in these processes and better understand how RBR function is modulated during transition from quiescence to division and from division to differentiation, we investigate RBR protein level and activity throughout different stages of auxin-induced lateral root formation. Lateral roots are initiated in non-dividing pericycle cells by auxin that first triggers their proliferation then later acts as an instructive signal for the execution of the lateral root developmental program. Similar to wild type, proliferation of the pericycle can also be elicited in several auxin transport mutants, however organized pericycle division and subsequent lateral root outgrowth relies strictly on established auxin gradient in the developing primordium. When auxin gradient cannot be realized (e.g. *pin*, *gnom* root) or pericycle division is induced by non-transportable auxin in the wild type, a disorganized primordium forms that fails to develop into lateral root. I present data showing how the RBR-E2F pathway operates under lateral root inducing conditions and that its mode of action is fundamentally different when lateral root formation is arrested at the disorganized primordium stage.

Our recent data also indicate a role for RBR that appears to be unrelated to the canonical RBR-E2F pathway. This function relies on the molecular scaffold property of RBR protein due to which RBR interacts with transcription factors and chromatin modifiers to modulate gene expression by chromatin remodeling. Data supporting such a role for RBR in lateral root formation will be presented.

## Potential signals for shifts in carbon allocation in response to nitrogen fertilisation

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The productivity of forests is partly determined by the amount of carbon that trees allocate to aboveground biomass, including wood, rather than belowground biomass, i.e. roots. The root to shoot ratio of plants is heavily influenced by the availability of nitrogen (N) in the soil. Addition of N to an N limited ecosystem results in a shift of plant biomass from belowground to aboveground, increasing production of wood and photosynthetic tissues. The control of this shift in allocation is not well understood. It is possible that the suppression of mycorrhizal fungi, possibly through the toxic effects of inorganic N application to the soil, may influence fungal signals to the plant and thus change allocation patterns. Alternatively, it is possible that carbon allocation is not controlled by a signal molecule, but simply by supply and demand: an N limited shoot cannot use photosynthetic carbon for growth, so the carbon is transported belowground, where it can be used to take up more N. With ample N, the shoot preferentially uses fixed carbon for growth, leaving less fixed carbon for the roots. We have circumvented any possible direct effects on soil organisms by adding N directly to spruce trees, by injection into the xylem. We report on the effects over two seasons, comparing plots with KNO<sub>3</sub> applied to the soil with plots where trees were directly injected with KNO<sub>3</sub>. We recorded changes in needle N content, CO<sub>2</sub> efflux from the soil (a combination of plant root and soil microorganism activity) and allocation patterns of <sup>13</sup>C within the plant after a pulse chase application of <sup>13</sup>CQ<sub>2</sub>. We report on the significance of changes in these factors with regard to potential signalling mechanisms.

## Poplar immunity at stake: new insights into the search for leaf rust effectors and their host targets

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Poplars are extensively cultivated for wood production but their susceptibility to the leaf rust fungus *Melampsora larici-populina* leads to considerable damages in plantations and impact their use as a crop tree. *Populus trichocarpa* and *M. larici-populina* were the first tree and rust fungal pathogen genomes sequenced making this pathosystem a model for post-genomic studies in forest pathology. Recent works identified molecular determinants likely required for the rust fungus to infect, grow and reproduce in poplar leaves. Biotrophic microbes modulate host physiology to their advantage by secreting effectors into infected tissues. In fungal pathogens, the few effectors described so far are lineage-specific small secreted proteins (SSPs) of unknown functions. Identifying the set of effectors and unravelling how they function to promote biotrophy is currently a major goal in phytopathology.

In *M. larici-populina*, 1,184 SSP-encoding genes have been identified which constitutes a great reservoir of putative effectors. Genomic organization into gene families, selection patterns, homology with known effectors, expression and localisation in infected poplar leaves were scrutinized to define a restricted list of ~20 candidate effectors now investigated at the functional level. A system for secretion of rust candidate effectors by *Pseudomonas syringae* into *Arabidopsis* leaves coupled to in planta bacterial growth monitoring showed that some of them have virulence functions. To further characterise these effectors, we focus our attention on the identification of their plant proteins targets. Some effectors have been produced as recombinant proteins and are now under biochemical and structural investigations. In planta-localisation studies have been initiated to determine whether effectors internalise into plant cells. Our understanding of how *M. larici-populina* promote its biotrophic lifestyle will ultimately help defining new strategies to fend off rust diseases.

## **Defence chemicals in European aspen and associated arthropod community structure**

**Benedicte Albrechtsen**

UPSC, Umeå University

*Abstract will come later*

## **When iron meets light : regulation of iron homeostasis by light, circadian clock and oxidative stress**

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Iron is a transition metal of particular importance for all living organisms. Due to its ability to gain and lose electrons, it is involved in many fundamental processes involving electron fluxes, like respiration or photosynthesis for example. However, free iron may chemically react with oxygen to form reactive oxygen species (ROS). Thus, iron homeostasis needs to be tightly regulated, to avoid starvation that may impair metabolism, and excess that may impair cell integrity. In all kingdoms, ferritins play a central role in the regulation of iron homeostasis. Ferritins form a hollow sphere able to accommodate thousands of iron atoms in a safe form unable to react with oxygen.

In order to get further insights into signaling pathways and molecular events controlling iron homeostasis in plants, we developed molecular and genetic approaches. We used the Arabidopsis ferritin gene *AtFer1* as a model gene. *AtFer1* is regulated by iron at the transcriptional level. We performed a genetic screen using *AtFer1* promoter region fused to the luciferase reporter gene, and isolated mutants affected in their response to iron. By positional cloning, we identified TIC (Time for Coffee) as a negative regulator of *AtFer1* expression. Interestingly, TIC was previously described as a regulator of the circadian clock and involved in responses to light, suggesting a link between the regulation of iron homeostasis, light and the circadian clock. To get further insights into such regulations, a yeast one hybrid screen, using several parts of *AtFer1* promoter region, allowed us to isolate *AtPHR1* and *AtPIF7* transcription factors. *AtPHR1* was previously identified as a phosphate starvation signaling element, and *AtPIF7* was shown to be involved in the circadian regulation of *DREB1* gene. The molecular links between iron, phosphate metabolism, light and circadian clock are currently under investigation, and first results on this field will be presented.

## **Plastidial glutaredoxins: glutathione-dependent enzymes involved in detoxification, redox signalling and iron-sulfur cluster biogenesis**

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Glutaredoxins are small oxidoreductases structurally related to thioredoxins. They have two major proposed biochemical roles, the reduction of disulfide bonds or the binding of iron-sulfur clusters both of which involve glutathione. In photosynthetic organisms, glutaredoxins are distributed into six classes. Some glutaredoxins from classes I and II can indeed exist either as apofoms which display deglutathionylation activity or as holoforms which bind [2Fe-2S] clusters. Using some biochemical, spectroscopic and structural approaches, we have characterized four plastidial glutaredoxins, showing that three of these can bind an iron-sulfur cluster. Site-directed mutagenesis experiments and resolution of the X-ray crystal structure of several glutaredoxins revealed the critical role of some cysteine residues for cluster formation and for protein activity. The deglutathionylation activity of the two plastidial class I glutaredoxins proceeds through a monothiol mechanism, which is important for the regeneration of two families of thiol-dependent antioxidant enzymes, namely thiol-peroxidases and methionine sulfoxide reductases. Finally, we have proposed that GrxS12, based on its peculiar catalytic and thermodynamic properties, could act as a redox sensor allowing glutathione to play some signaling functions.

## **Ethanol production from lignocellulose using a filamentous biocatalyst**

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Ethanol production by the biocatalyst *Trametes versicolor* was characterized from lignocellulose. It was shown that *T. versicolor* can ferment xylose efficiently to ethanol in media containing mixtures of hexoses and xylose, yielding ethanol concentrations of 20.0 g/l. Very strong correlations were found between ethanolic fermentation (alcohol dehydrogenase activity and ethanol production), sugar consumption and xylose catabolism after 354 hours in culture. In a medium containing a 1:1 glucose:xylose ratio, fermentation efficiency of xylose into ethanol was 80% after 354 hours. Inhibitors were tolerated to a large extent by the biocatalyst. Results are discussed in relation to biotechnological relevance.

## **Complexity and conservation of regulatory networks in plants**

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Systems biology is about describing how properties of biological systems emerge from networks of interacting genes, proteins and metabolites. We are particularly interested in understanding how one can perturb such networks to engineer trees with improved properties such as increased biomass production. We are developing approaches to reverse-engineer regulatory networks from omics data and methods to compare such networks across species. We apply network inference to transcriptional data from aspen leaves and wood, and network comparison to compilations of transcriptional data across aspen, rice and Arabidopsis. We find interesting patterns related to the regulatory complexity in aspen, such as the extent of synergistic interactions, and the nature of network conservation across plants, such as the high conservation of network neighbourhoods compared to individual interactions. In practical applications, we are currently using networks to interpret observed phenotypes in transgenic aspens and to identify targets for new transgenic trees.



## Evolution and modeling of the LEAFY DNA binding specificity

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Changes in transcriptional networks represent an efficient way to create evolutionary novelties but they are often difficult to predict directly from genome sequence. We study the evolution of the network orchestrated by the LEAFY protein. This transcription factor is unique to plants, it does not resemble any other protein and does not belong to a multigene family. It is a master regulator of flower development in angiosperms but is also present in non-flowering land plants such as mosses or gymnosperms. It thus constitutes a good opportunity to study transcription factor evolution in plants. To gain insight into how this factor works, we have solved the crystallographic structure of its DNA binding domain, revealing a novel protein fold in contact with a large region of DNA. Using this knowledge together with a biochemical characterization, we built a biophysical model that predicts LFY DNA binding in vitro and in vivo (in the *Arabidopsis thaliana* genome). Because LEAFY specificity is highly conserved in flowering plants, we could use the model to analyze the evolution of the link between LEAFY and some of its targets directly from angiosperms genomic sequences. As opposed to angiosperms, the situation is different in the moss *Physcomitrella patens*, where LEAFY displays a distinct DNA binding specificity despite of the extensive conservation of the residues binding DNA. We will present our attempts to understand how a conserved and essential factor can change specificity over evolution.

## A data-driven integrative model of sepal primordium polarity in *Arabidopsis*

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Flower patterning is controlled by a complex molecular network but how this network functions remains to be elucidated. Here, we developed an integrative modeling approach allowing the assembly of heterogeneous data into a biologically coherent model, which allows predictions to be made and inconsistencies among the data to be found. We used this approach to study the network underlying sepal development in the young flower of *Arabidopsis*. We constructed a digital atlas of gene expression and used it to build a dynamical molecular regulatory network model of the sepal primordium development. This led to the construction of the first coherent molecular network model underlying lateral organ polarity, which fully recapitulates expression and interaction data. Remarkably, our model predicts the existence of three novel pathways involving the HD-ZIP III genes and both cytokinin and ARGONAUTE members. In addition, our model provides predictions on molecular interactions. In a broader context, this approach allows the extraction of biological knowledge from diverse type of data and can be used to study developmental processes in any multicellular organism.

## Sequencing and assembly of the Norway spruce (*Picea abies*)

The Spruce genome project; **Nathaniel Street**

Conifers are the dominant plant species in many ecosystems, including large areas in Sweden. Despite this, no conifer genome has yet been published, mainly owing to their large size and complexity. The lack of a genome sequence has hampered our understanding of conifer biology and evolution, as well as the development of potential novel breeding strategies of these economically important species.

We are currently performing whole genome sequencing and assembly of the 20 Gbp Norway spruce genome. This genome contains huge amounts of repeated elements, with an estimated gene density of only 1/500 kbp. In common with other tree genomes, heterozygosity is high, which further complicates the assembly process. The Spruce Genome Project is addressing questions of genome size, content and evolution, including analyses of gene families and repeats, and will establish Norway spruce as a prime model species for conifer research.

I will present our main strategies concerning sequencing and assembly of the Norway spruce genome, and give an update on the results obtained so far. In brief, we use a combination of whole genome shotgun and fosmid pool sequencing, followed by scaffolding and merging of the separate assemblies. This is complemented by a manually curated spruce-specific repeat library, sequencing of random fosmid clones for assembly benchmarking, as well as assemblies of the chloroplast and mitochondrial genomes.

By this approach we hope to rapidly achieve an accurate and comprehensive genomic resource that will be of great value to the conifer, and wider plant, community.

***Poster***

## Plasticity of genes expression in response to drought in poplar growing leaves

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In the context of climatic changes, perennials species will have to face changes in water availability and the sustainability of actual populations relies on individual accommodation capacities. Accommodation capacities partly depend on phenotypic plasticity which describes the capacity of a genotype to modify its phenotype in response to changes of environmental conditions. Under water deficit, limiting the increase of leaf surface area contributes to limit water losses due to transpiration and thus can be viewed as adaptative phenotypic plasticity. Indeed, leaf growth, which depends on cell production and cell expansion, is one of the first parameters to be impacted by water deficit.

This study aims at analyzing phenotypic plasticity at the molecular scale in growing leaves of poplar, in response to drought and to reirrigation. We focused on the expression level of some selected genes involved in cell expansion: expansins, xyloglucan endotransglucosylases, pectin methylesterase and aquaporins. Expression levels were quantified by qPCR in three leaves of contrasting maturity stages and growth rates (juvenile, intermediate and mature), in four poplar genotypes: three *P. deltoides* x *P. nigra* and one *P. deltoides* x *P. trichocarpa*. As expected, most of these genes were upregulated in growing leaves as compared to mature leaves. Similar patterns were found for gene expression levels and growth parameters and/or drought tolerance of the different genotypes. Genes were also upregulated in the fastest growing leaf (intermediate) in response to both drought and reirrigation for three out of four genotypes, while expression levels were mainly not affected in the juvenile and mature leaves. Overall, growth and expression levels were not restored after three days of reirrigation. Our results highlight a strong phenotypic plasticity of gene expression, which differs qualitatively among genotypes and among leaf maturity stages within genotypes.

**PhD project:****Cell division and cell elongation in the growing root apex: diversity of drought-induced responses**

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The great adaptability to the environmental constraints of root development is a crucial property in tree functioning. Root system development, including root growth and lateral root formation, is responsive to numerous factors of the rhizosphere, including interactions with fungi, nutrient availability, and water availability. Cellular and molecular mechanisms responsible for this developmental plasticity in trees are almost unknown as well as the diversity of this plasticity. This diversity could reflect different strategies of drought tolerance. At a physiological scale, root growth relies on two dynamic processes: the meristematic production of cells and their elongation. Both are responsive to environmental factors. While the molecular mechanisms underlying cell division and cell elongation have been depicted, those involved in the synchrony between these cellular processes remain puzzling. Moreover, the molecular underpinnings of how the environmental cues are transmitted into plant level phenotypic changes (e.g. lateral root proliferation and root growth) have been hardly discovered in *Arabidopsis* and are still overlooked in trees.

In this context, the main objectives of my PhD project are the following:

1. Studying the diversity of root growth response to drought at a cellular scale in poplar with a kinematic approach, by giving much attention to growth transition phases between different water availability.
2. Combining kinematic approach with gene expression monitoring and in situ analysis to decipher the spatial and temporal regulation of cell division and cell elongation, and identify molecular mechanisms involved in the synchrony between those cellular processes.
3. Coupling these tools with a modelling approach to highlight different strategies of drought tolerance in various *Populus* species and to understand underlying mechanisms.

## Elucidation of the function of an alternatively spliced gene in vascular development in *Arabidopsis*

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Elucidation of a function of important genes is a priority of a post-genomic research. By carrying out a survey of genes expressed during vascular development, we have discovered an alternatively spliced gene of totally unknown function, called GENE A. The introduction of a copy of this gene to the *Arabidopsis* genome caused a striking phenotype. The reason for such phenotype and the role of the alternative splicing of the gene A are not known. Alternative splicing is a mean of generation of variety of functional adaptations based on one gene locus. It is a common mean to regulate function of genes in animals, where most genes are alternatively spliced. In plants, the alternatively spliced genes are poorly characterized but the known examples of them include genes of important regulatory functions in development, stress and hormone responses. The implementation of a new method using induced xylogenesis in transformable *Arabidopsis* suspensions as well as the observation of the phenotypes of changed expression of the gene A and its variants bring support for the importance of gene A expression for plant development and xylogenesis.

## **NRT1.1 spatio-temporal expression pattern confirms its implication in lateral root development of *Arabidopsis thaliana* seedlings**

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Nitrate ( $\text{NO}_3^-$ ) is a major nutrient for plants strongly affecting the root system architecture and more particularly lateral root development. In *Arabidopsis thaliana*, NRT1.1 acts both as a  $\text{NO}_3^-$  transporter and a  $\text{NO}_3^-$  sensor. In *nrt1.1* K.O. mutant there is an abnormal lateral root proliferation under  $\text{NO}_3^-$  deficiency correlating with an abnormal auxin accumulation in lateral root primordia. AtNRT1.1 was shown to facilitate auxin uptake in heterologous systems (*Xenopus* oocytes and BY2 cells) and GFP tagged proteins showed its expression pattern was restricted to the epidermis of lateral root primordia. Based on these data and according to the auxin fountain model (Benkova et al., 2004), NRT1.1 was proposed to contribute to the epidermis mediated auxin basipetal flow under  $\text{NO}_3^-$  deficiency leading to a local depletion which in turns represses lateral root development (Krouk et al. 2010).

According to this model, NRT1.1 should be expressed earlier and stronger in lateral root primordia of seedlings grown on 0mM than on 1mM  $\text{NO}_3^-$ . On the contrary LAX3 expression (an auxin-induced auxin influx transporter involved in cell wall loosening in primordia surrounding cells) should be negatively correlated.

Here, we studied the kinetics of NRT1.1 promoter activity, and NRT1.1 and LAX3 proteins expression pattern in response to  $\text{NO}_3^-$ . Since NRT1.1-GFP signal is highly sensitive to photobleaching, a statistical approach was preferred to timelapse imaging. Our results are consistent with the model.



## Developmental analysis of Mediator mutants in *Arabidopsis*

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Mediator is a multi-subunit complex originally identified in yeast. It is required for activator-dependent stimulation of RNA polymerase II (Pol II) transcription (Kelleher et al., 1990) by acting as a bridge between DNA binding Transcription Factors (TFs) and the general Pol II machinery. The *Arabidopsis* Mediator was recently biochemically characterized and contains 21 subunits with yeast or metazoan homologs and 6 more “plant-specific” subunits (Bäckstrom et al., 2007). Some *Arabidopsis* genes encoding Mediator subunits had been characterized earlier and showed different phenotypes like embryo lethality (Dhawan et al., 2009), delayed flowering (Cerdan and Chory, 2003), salt resistance (Kim et al., 2011), and pathogen resistance (Kidd et al., 2009). These results indicated that Mediator integrates various signaling pathways at the molecular level (Bäckstrom et al., 2007). Regulation of gene expression at the transcriptional level is essential for all cellular and developmental processes in plant. Around 1,500 TFs have been identified in *Arabidopsis* suggesting that different TFs could potentially interact with the same Mediator subunit to integrate different pathways and to adjust the cellular response according to the external stimuli. In this perspective, the systemic analysis of loss-of-function mutants of different mediator subunits should allow identification of subunit involved in different processes. We have characterized 17 different mutants in long day conditions by measuring flowering time, number of branches, size of some organs, secondary growth and root growth. We have coupled this phenotypic characterization with a metabolomic approach to correlate phenotypes with metabolic pathways. Our phenotypic analysis matches well with the metabolomic results. Indeed the mutants presenting a difference in the metabolomic profile seem also to show a developmental phenotype. We have found 3 mutants that show a delay in flowering time, which correlates with a different metabolite profile. We have also characterized a mutant involved in an early senescence process. Moreover some of these mutants also show a resistance or sensitivity to abiotic/biotic stress. This suggests that the Mediator subunit involved can regulate different pathways and to function as an integrator of external signals in this development process

## RMS3, an $\pm^2$ hydrolase involved in the strigolactone perception

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The study of shoot branching in pea, using the high branching ramosus (rms) mutants has highlighted the existence of a new family of plant hormones: the strigolactones, inhibiting shoot branching in seed plants (Gomez-Roldan V. et al., 2008 Umehara 2008). The discovery of this novel plant hormone opens a new possibility in the understanding of plant development, especially in the strigolactone perception. This work concerns the rms3 pea mutant, which is highly branched (Beveridge et al. 1996) and which doesn't respond to strigolactones.

We have shown recently the role of the pea TCP transcription factor, PsBRC1 homolog to the maize TEOSINTE BRANCHED (TB1) in the response to strigolactones (Braun et al., 2011). We are currently investigating and characterizing other elements in the signaling pathway, including the strigolactone receptor. In pea, two other mutants do not respond to the application of strigolactones, rms3 and rms4. The RMS4 gene encodes an F-BOX protein (Johnson et al. 2006) and the RMS3 gene has not yet been cloned.

Here we show that RMS3 is the homolog of the rice D14 gene encoding a protein of the  $\pm^2$ /hydrolase superfamily (Arite et al. 2009). GID1, the gibberellin (GA) receptor is also member of this family and RMS3 is a good candidate for the strigolactone receptor. With strigolactone applications and grafting experiments with different mutated alleles of rms3, we have shown that this protein was acting in the strigolactone response. We produced the RMS3 protein in the bacterial system *E. coli*, and we are currently testing its putative enzymatic activity and its binding with a radio-labeled strigolactone to determine the exact role of this protein in the strigolactone signaling pathway.

## **A new non floral function of LFY revealed by molecular analysis in *Arabidopsis thaliana***

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The transcription factor LEAFY (LFY) is a central regulator of floral development in angiosperms. In this group of plants, LFY confers a floral fate to emerging meristems through the transcriptional activation of floral organ identity genes. Nevertheless LFY is also present in other land plants, including non-flowering plants, suggesting that LFY must have non floral functions. Several lines of evidences in the literature suggest that LFY is involved in the formation of meristems in higher plants (Moyroud et al., 2010). Using a set of mutant and transgenic lines (created using the 3D structure of LEAFY DNA binding domain) as well as genomic chromatin immunoprecipitation, we show here that LFY transcriptionally regulates several genes involved in meristem initiation in the model plant *Arabidopsis thaliana*. We will present our latest results on this set of novel LEAFY targets and will discuss their role in the early steps of meristem formation, prior to floral determination.

## A WUSCHEL-like gene controls stem secondary growth in trees

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Wood formation starts with cell division and differentiation in a secondary meristem called vascular cambium, which forms a continuous cylinder of meristematic cells in the stem. Although many anatomical studies have been performed on the cambial zone and its derivatives, very little is currently known about the molecular and genetic mechanisms regulating the maintenance and differentiation of these stem cells as well as the patterning during the secondary growth of the woody plants. Here we investigate the role of a WUSCHEL-like gene PtHB3 during secondary growth in Poplar. In the transgenic plants expressing an RNAi construct targeting the PtHB3 gene, the width of the vascular cambium was severely reduced and the secondary growth was severely diminished, showing that PtHB3 controls the cell identity and division activity in the vascular cambium. Moreover, ectopic expression of a Poplar CLE41/44-like (CLAVATA3/ESR-RELATED 41/44) gene in trees caused defects in the establishment of cambial cell divisions and the patterning of the vascular tissues. Based on the transcriptional data, a positive feed-forward loop involving PtHB3, PtCLE41 and the receptor-like kinase gene PtRLK3 is suggested to regulate the identity and activity of the vascular cambium.

## Biochemical and functional characterization of poplar glutathione S-transferases containing a cysteine as a catalytic residue

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Glutathione S-transferases (GSTs) constitute a complex and widespread protein superfamily classified as enzymes of secondary metabolism. Regarding plants, beyond their well documented role in the detoxification of herbicides in annual species through glutathionylation reactions, it is only recently, that some GSTs were shown to bind to flavonoids, oxygenated fatty acids or porphyrins, thus pointing to their importance for diverse metabolic pathways. This might be related to the large diversity of sequences found in living organisms. Indeed, in plants, 50 to 90 genes (83 genes in poplar) are distributed in 7 classes with 2 classes being plant specific. Most of these roles are likely related to the capacity of GSTs to transfer a glutathione molecule to xenobiotics or secondary metabolites. However, this important diversity described at the genomic level raises the question of the function(s) of these enzymes and of their potential redundancy or specificity.

Most characterized GSTs possess Ser or Tyr as catalytic residues but some GSTs possess instead a cysteine in a CPx[A/C/S] sequence, resembling to glutaredoxin active site sequences. A phylogenetic analysis of *Populus trichocarpa* genome indicated that cysteine-containing GST proteins can be divided into four classes: (i) Lambda, (ii) Dehydroascorbate Reductase (DHAR), (iii) Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) and (iv) Glutathionyl Hydroquinone Reductase (GHR).

The aim of this work is to investigate the biochemical and functional properties of these four classes. Recombinant proteins, as well as some mutated proteins on cysteine catalytic residues, have been expressed in *Escherichia coli* and purified. Biochemical studies with various substrates showed that these classes possess specific substrates and catalytic properties that differ from other GSTs having a serine as a catalytic residue. On the other hand, their subcellular localisation has been studied through the transient expression in tobacco of translational fusion proteins with GFP.

## Identifying molecular sugar responses in *Arabidopsis*

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Growth and development of a plant requires precise control and coordination mechanisms of sugar metabolism. The aim of this project is to understand how plants respond to changes in sugar levels at the molecular level. *Arabidopsis* seedlings were grown in liquid medium with sucrose and then depleted of carbon before sucrose was resupplied. Soluble proteome derived phosphopeptides were analyzed quantitatively by mass spectrometry over five time points of sucrose resupply. This analysis identified a protein we named Sucrose C-terminus Dephosphorylated protein (SCDP). A carboxyterminal serine of SCDP was rapidly dephosphorylated in response to sucrose, glucose and fructose. Null mutants of *scdp* showed a stunted growth phenotype indicating a role for SCDP in plant growth and development. In our second approach we have used forward genetics to screen for suppressors of *cob-2*. *cob-2* has an amino acid change in the COBRA protein, which is essential for cellulose biosynthesis. *cob-2* is a conditional mutant showing a root swelling phenotype only in the presence of exogenous sugars. The mechanism of this sugar inducible phenotype is not known but may be linked to regulation of sugar homeostasis and sugar flux to cell walls. We screened an EMS mutagenised population of *cob-2* and identified five mutants in which the sugar response was suppressed. Two of the strongest suppression lines were selected for mapping.

## **Microtubules and secondary cell wall patterning in xylem vessels**

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## Heterologous expression of fungal acetyl xylan esterase (CE1) in *Arabidopsis*

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Xylan is the third most abundant biopolymer found on the Earth and it contributes to a large amount of biomass available for human exploitation. Xylan backbone consists of  $\beta$ -(1-4) linked D-xylopyranosyl residues substituted with 4-O-methyl-D-glucuronic acid/glucuronic acid. The xylopyranosyl residues are partially acetylated at the C-2 and/or C-3 positions. Xylan acetylation might affect the conversion of lignocellulosic biomass to fermentable sugar, which is a crucial step in biofuel production, and it is important for xylan physicochemical properties. Our aim is to understand intricate mechanism of xylan interactions in cell wall and to develop plants with improved characteristics for the production of biofuel and cost effective processing of biomass. In our project, we have overexpressed fungal acetyl xylan esterase (CE1) in *Arabidopsis* to modify xylan acetylation. It was possible to overexpress CE1 in *Arabidopsis* with no major visible morphological effect. We found reduced acetylation in the transgenic lines as compared to WT by FTIR and MALDI. Saccharification was also improved in the transgenic lines as compared to WT.



## **Somatic embryo development in maritime pine (*Pinus pinaster*): a 2-DE proteomic analysis**

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Development of clonal propagation method, such as somatic embryogenesis (SE) has potentially numerous applications such as the production of a large number of genetically improved plants. However it is necessary to optimise somatic embryo development, which remains difficult in pine species. Since the early protocols of SE were developed for spruce species (*Picea*), it was assumed that they would be applicable to pines; however, it became apparent that pines were less responsive. One limitation to large-scale propagation of majority of commercial pine species through SE is the lack or low somatic embryo maturation efficiency, which result in the inability to capture certain families. This has caused great concerns among breeders and foresters of potential adverse selection pressure.

Current maturation protocols lead to a development of mature somatic embryos that morphologically resemble zygotic embryos. These somatic embryos are harvested after arbitrarily chosen periods of time and are germinated and further grown in a greenhouse. This procedure only gives an indication that the maturation was successful if produced somatic embryos were able to convert to plants. Such an empirical approach does not give any information of the quality of somatic embryos with respect to storage reserves accumulation nor does it gives information on the optimal time for harvesting to achieve maximal plant conversion rates. Therefore, there is a need to develop markers that could be used for quality control of different batches of somatic embryos that are matured, or when different maturation protocols are applied. Storage protein accumulation has been followed and the identity of the proteins accumulated by the somatic embryos has been tested by 2D gel electrophoresis.

Final objective is to have a better understanding of the maturation of *Pinus pinaster*. The description of SE would contribute to optimise the maturation process and the in vitro production of plants

## **Roles of very-long-chain fatty acid in the development of *Arabidopsis thaliana***

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Very long chain fatty acids (VLCFAs) are necessary for the synthesis of triacylglycerols, epicuticular waxes and sphingolipides which are well known to be essential for seed storage and plant structure. Interestingly, studies showed that VLCFAs are also essential for plant development being involved in several cellular processes such as membrane trafficking, cell division and cell differentiation (1-3). The VLCFAs are elongated in the endoplasmic reticulum by the elongase complex composed of four enzymes. The acyl-CoA dehydratase involved in the third step was recently identified as PASTICCINO2 (PAS2) (4). The *pas2* mutants show strong defects such as lost of cellular adherence, defects in division plate formation and vesicular dynamic (1, 4).

However, the precise role of VLCFAs in these different cellular processes is still poorly understood in plants. In order to identify new factors associated with the biosynthesis or function of VLCFAs, a yeast multicopy suppressors screen with an *A. thaliana* cDNA library was carried out in a yeast mutant strain defective for fatty acid elongation. Loss of function of PHS1, the yeast PAS2 ortholog, prevents growth and induce cytokinesis defects. We selected *Arabidopsis* genes able to restore growth in selective conditions by acting either directly on the VLCFAs synthesis or by by-passing the VLCFA requirement for cell division and growth. Results from the screen will be presented and in particular the identification of potentially a new dehydratase involved in VLCFAs elongation.

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## Tissue specific profiling of the *Arabidopsis thaliana* auxin metabolome

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The hormonal profiling methods use modern analytical tools based on fast chromatographic separation and mass spectrometric quantitative analysis. The plant hormone auxin is believed to influence almost every aspect of plant growth and development. We have developed and validated a method for profiling the majority of known auxin precursors and conjugates/catabolites in small amounts of *Arabidopsis* tissue. Our method includes trace analysis of 21 compounds with different polarity, acidity and basicity as well as stability and abundance in crude plant extract. We found that a using of polymer based (Oasis HLB) sorbents was the best tool in the one-step purification, including a new derivatization method to quantify the most labile of the auxin precursors. The process was completed by a single chromatographic analysis of auxin metabolites in 12 minutes using an analytical column packed with sub-2-microne particles. In multiple reaction monitoring mode, the detection limit for most of analytes ranged from 1.0 to 5.0 fmol and achieved linear range was at least five orders of magnitude. Finally, we have profiled the auxin metabolome in root and shoot tissues from different *Arabidopsis thaliana* ecotypes and auxin overproducing mutant lines, showing substantial differences in the metabolite pattern between the lines and between different tissues. We also observed differences in abundance of several orders of magnitude between different auxin metabolites, indicating the relative importance of different auxin precursors and conjugates/catabolites for maintaining auxin homeostasis. We now have a powerful tool to get a better understanding of the regulation of auxin metabolism during plant development.

## **MYB103 regulates FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in *Arabidopsis* stems**

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The transcription factor (TF) MYB103 has earlier been identified as a member in the transcriptional network regulating secondary wall biosynthesis in xylem tissues of *Arabidopsis*. It is a direct transcriptional target of the NAC TF SND1, and expression of 35S-driven dominant repression or over-expression MYB103 constructs modifies secondary wall thickness. We identified two *myb103* T-DNA insertion mutants and chemically characterised their lignocellulose by pyrolysis-GC/MS, 2D NMR, FT-IR microspectroscopy and wet chemistry. The mutants developed normally but exhibited a large change in their cell wall chemistry, marked by a 70–75% decrease in syringyl (S) lignin. Guaiacyl lignin was co-ordinately increased, so that total Klason lignin was not affected. Transcript abundance of FERULATE-5-HYDROXYLASE (F5H), the key gene in S-lignin biosynthesis, was decreased by 70–75% in the *myb103* mutants, and the metabolome of the *myb103* mutant and of a null mutant in F5H was very similar. This shows that F5H expression is dependent on MYB103. Microarray analysis revealed that many other TFs putatively involved in secondary wall biosynthesis were also down-regulated in the *myb103* mutants. However, a protoplast transactivation assay did not show any strong interaction between MYB103 (or other down-regulated TFs) and the F5H promoter. In conclusion, we demonstrate that MYB103 regulates F5H expression and S-lignin biosynthesis in *Arabidopsis*.

## **The Pickle-Link: RBR1 mediated chromatin remodeling during lateral root formation in *Arabidopsis***

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Lateral root (LR) formation is an example of post-embryonic plant development when a new organ arises from an already differentiated tissue. Non-dividing pericycle cells that will give rise to a new lateral root must re-enter the cell cycle and regain cell division activity then finally differentiate into distinct cell types that build up the new organ. During developmental phase transitions like cellular dedifferentiation the cell's existing developmental information must be erased and reprogrammed to make cells responsive to new signals and dedifferentiation is accompanied by structural reorganization of the chromatin that brings about the necessary changes in gene expression pattern.

It is known that auxin signals converging distinct pericycle cells promote degradation of Aux/IAAs proteins (SLR/IAA14 etc.) involved in LR initiation. Elimination of Aux/IAA repressors through SCFTIR1/AFBs ubiquitin ligase complexes and the 26S proteasome results in activation of ARF7/19 functions thereby allowing ARF7/19 transcription factors to drive expression of LBD16, LBD29 and other target genes required for auxin response and LR initiation. LR formation is compromised in the *slr-1* gain-of-function mutant expressing a stabilized, non-degradable version of the SLR/IAA14 protein. However, mutation of the PICKLE gene, encoding a CHD3/Mi-2 chromatin remodeling factor restores LR formation in the *slr-1* background. This findings indicates that in addition to the well-established auxin/ SCFTIR1/AFBs/SLR/ARF LR initiation pathway additional functions with roles in chromatin remodeling are needed and are acting in concert with hormone signaling.

Supported by our preliminary data we assume that chromatin remodeling plays a crucial role in lateral root formation and the retinoblastoma-related protein 1 (RBR1) is at the core of remodeling complexes.

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## **Auxin signaling and growth rhythmicity at the shoot apical meristem**

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The shoot apical meristem, composed of a small group of undifferentiated cells, generates all the aerial parts of the plant that arise after germination. Lateral organs (leaves and flowers) initiated at the SAM, emerged in a very precise spatio-temporal pattern called phyllotaxis. This process requires a tight regulation of cell identity and morphogenesis in space and time. It has been now well described that auxin is a key signal in controlling SAM development. Indeed, auxin accumulation at the meristem periphery is sufficient to trigger organogenesis.

We developed a new auxin signaling sensor, called DII-VENUS, which is composed of the fusion of the domain II of IAA28 to a fast-maturing YFP targeted to the nucleus, and expressed under control of the ubiquitous 35S promoter. By its design, DII-VENUS can monitor with a cellular resolution local degradation of Aux/IAAs that directly depends on auxin levels. We used this tool to investigate the distribution and dynamics of auxin in a growing meristem. Interestingly, we found that auxin levels in the SAM fluctuate with a surprising regularity (with a ~24 hours period) and independently of auxin polar transport since auxin fluctuations are also detected in pinoid and pin-formed1 mutant. We also observed that auxin fluctuations in the SAM are maintained under different light conditions suggesting that this process is probably controlled by an endogenous clock. We provide preliminary evidence that these rhythmic hormonal pulses in the structure correlate with growth maxima in the meristem, suggesting a dual function of auxin in organ initiation and global growth regulation in the meristem.

## **Characterization of auxin-related genes network(s) involved in regulating the earliest steps of adventitious root initiation in *P. trichocarpa* (easy-to-root) and *P. tremula* (difficult-to-root) species**

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Genetic studies indicate that the competence to form adventitious roots (ARs) is a quantitative genetic trait. In woody species, competence to form ARs is quantified as percentage of rooted cuttings and economically important genotypes of apple, eucalyptus, poplar, pine, or other woody species are classified as easy- or difficult-to-root. Nevertheless the molecular mechanisms underlying this variability are still largely unknown.

In the recent years, Bellini's group has identified, through the characterization of *Arabidopsis* mutants altered in their aptitude to produce adventitious roots, several genes including three transcription factors from the Auxin Response Factor family (AtARF6, 8 and 17) and their downstream target from the auxin inducible GH3 gene family (AtGH3.3, AtGH3.5 and AtGH3.6) that are involved in the control of AR initiation. Moreover, using genomics approach, we have identified the transcriptional profiles associated with the different developmental stages of adventitious root formation in the model tree species *Populus* (V. Legué's oral presentation).

Taking advantage of the knowledge acquired until now in *Arabidopsis* and poplar, the aim of this work, developed in the frame of UPRA (UPSC/INRA cooperation), is to investigate the role of the candidate genes in regulating the earliest steps of ARs initiation in *P. trichocarpa* (easy-to-root) and *P. tremula* (difficult-to-root) species, through different approaches. We are obtaining transgenic poplar lines overexpressing or silencing PtrARF8;1 and PtrARF17;1 transcription factors together with promoter:GUS lines, in order to study function and expression pattern of these ARFs. In parallel, we are investigating the putative role of candidate genes by comparing their expression in the two different poplar species, during the AR initiation in stem cuttings. Moreover, we are characterizing the adventitious rooting process in the two genotypes through rooting assay in hydroponic and in vitro conditions and morphological observations by use of light microscopy.

## **Fructokinase is required for carbon partitioning to cellulose in aspen wood**

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Sucrose is the main transported form of carbon in several plant species including the model tree aspen. Sucrose metabolism in developing wood is therefore central for the regulation of carbon partitioning to stem biomass. Half of the sucrose-derived carbon is in the form of fructose, but metabolism of fructose has received little attention as a factor in carbon partitioning to wood cell walls. We have identified a fructokinase isoform (FRK2), which is important for fructose phosphorylation and carbon flux to cellulose in aspen. RNAi mediated reduction of FRK2 activity in developing wood led to accumulation of soluble neutral sugars and a decrease in hexose phosphates and UDP-glucose indicating that carbon flux to the cell wall polysaccharide precursors was decreased. Reduced FRK2 activity also led to thinner fiber cell walls with a reduction in the proportion of cellulose, while having no major effect on hemicelluloses. No pleiotropic effects on stem height or diameter growth were observed. The results establish a central role for the FRK2 activity in carbon flux to wood cellulose.



## **The mechanism of tracheary element lignification in *Arabidopsis thaliana* cell cultures**

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Lignin is a phenolic polymer deposited in the cell wall of specialized cells such as xylem tracheary elements (TEs) [1] which form the water and mineral conducts of the land plants vascular system. Lignin has multiple functions as it provides additional mechanical support to plant organs, cell wall impermeability and a structural barrier against pathogens [2]. In this study we are using the *Arabidopsis* in vitro TE differentiation system in which lignified TEs can be induced by adding hormones [3]. Recently, live cell imaging of TE formation showed that lignification occurred after TE programmed cell death (PCD) suggesting that lignin results from a distinct and novel mechanism [4]. We confirmed that lignification occurred effectively after PCD by pharmacologically inhibiting TE-PCD or lignin monomer synthesis. Quantification of phenolic compounds revealed an increase of both intracellular and extracellular phenolics in cultures undergoing TE differentiation and an over-accumulation when pharmacologically inhibiting TE-PCD or lignin monomer synthesis. The sub-fractionation of phenolics showed that the accumulated phenolics are mainly glycosides. As only 50% of cells transdifferentiate into TEs, TEs were separated from non-TE cells to define if lignin monomer synthesis was confined to TEs or not. Gene expression analysis showed that lignin monomer synthesis genes are expressed in both TEs and non-TE cells. These results suggest that lignin synthesis is a partially

## Unravelling carbon allocation to woody biomass in aspen

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The aim of this project is to understand carbon flux from photosynthetic tissues to wood biosynthesis in aspen. We are using following three approaches: (1) To monitor the rate of carbon transport as well as the synthesis and deposition of different cell wall polymers in developing wood we are using  $^{13}\text{CO}_2$  pulse-chase labelling. We discovered that already after 2 hours of  $^{13}\text{CO}_2$  supply  $^{13}\text{C}$  could be found in the sucrose pool of aspen wood. The  $^{13}\text{C}$  was detected in the cell walls after 12 hours. Interestingly  $^{13}\text{C}$  accumulated in the cell walls up to 72 hours after the end of a 6-hour  $^{13}\text{CO}_2$  pulse suggesting the presence of intermediary carbon storage pool/s in the leaf to wood pathway. (2) Wood ray cells represent a likely lateral carbon transport route, to better understand ray cell function we isolated RNA from ray cells using laser microdissection. The RNA is being sequenced alongside the wood developmental gradient samples to provide a spatial understanding of ray cell specific transcripts. (3) Micro-scale analysis of metabolites and enzyme activities across the tangential gradient of wood development. This high-resolution profile will reveal the soluble carbohydrate status and associated enzyme activities across the phloem, cambium, xylem differentiation and expansion, and secondary wall deposition zones of the developing wood. This analysis will serve to identify key enzyme activities associated with each stage of wood development, and thus pinpoint potential routes of carbon flux to wood cell walls.

## Manipulation of root system architecture by the ectomycorrhizal fungus, *Laccaria bicolor*: control of auxin pathways

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The soil environment of plant roots contains a diverse and complex community of microorganisms. Some of them can interact with root to form a new organ in which a mutualistic exchange of nutrients occurs including ectomycorrhizae. The succession of events in the symbiotic interaction between tree roots and ectomycorrhizal fungi are accompanied by a modification of root architecture including a stimulation of lateral root development followed by an inhibition of root growth. It has been demonstrated that the phytohormone auxin is one of the main triggers regulating lateral root formation, and it plays a role in the interaction between a microorganism and its host. A recent study has implicated auxin in signalling to alter root system architecture during the establishment of ectomycorrhizal symbiosis between poplar and the ectomycorrhizae fungus *Laccaria bicolor*. A global transcriptomic approach suggested that auxin transport and signalization are involved in modification of root architecture in response to *Laccaria bicolor*. Transcripts of PtaPIN such as PtaPIN9, an orthologue of AtPIN2, involving in auxin efflux transport, are modulated during the interaction between the two partners. Functional analysis of these candidate's genes are being investigated. Interestingly, *L. bicolor* did not induce lateral root stimulation in transgenic *Populus* overexpressing PtaPIN9 and RNAi mediated down-regulation of PtaPIN9 expression. The immuno-localisation of PtaPIN is investigating in poplar ectomycorrhizae roots. This data will allow us to describe the impact of auxin pathway during fungus/root cross talk.