# Auxins and Cytokinins in Plant Development

... and Interactions with Other Phytohormones

# International Symposium 2014

# BOOK OF ABSTRACTS

## CONTENT

# PLENARY LECTURES

## Plenary Lecture 1

### Auxin perception and response in Arabidopsis and moss

**Mark Estelle[1], Rammyani Bagchi[1], Bastiaan Bargmann[1], Cristina Castillejo[1], Silka Cheng[1], Goh Choe[1], Meirav Lavy[1], Dior Kelley[1], Kerstin Kirchsteiger[1], Mike Prigge[1], Mohammad Salehin[1], Stephanie Shain[1], Sibo Tao[1], Renhou Wang[1], Hong Yu[1], Yi Zhang[1]**
*[1]University of California San Diego, La Jolla CA, USA*

E-mail of presenting author: mestelle@me.com

Auxin regulates a bewildering array of processes during plant growth and development. This complexity contrasts with the apparent simplicity of the auxin-signaling pathway. Auxin regulates transcription through the TIR1/AFB-Aux/IAA-ARF pathway. The hormone directly binds to the F-box protein of the $SCF^{TIR1/AFB}$ ubiquitin protein ligase E3 and promotes an interaction with the Aux/IAA transcriptional repressors, thus stimulating their degradation. Loss of the Aux/IAAs permits ARF-dependent transcription. In the case of the TIR1/AFB proteins, recent results indicate that different members of the family have distinct activities both with respect to auxin binding and Aux/IAA interaction. We are currently exploring the possibility that these differences contribute to the complexity of auxin response. In addition we have used the yeast 2-hybrid system to isolate *tir1* mutants that illustrate novel aspects of TIR1 function and regulation. Finally, we are investigating the downstream transcriptional networks that mediate auxin-dependent growth responses.

## Plenary Lecture 2

### Cytokinin: Beyond Two Component Signaling

**Joseph Kieber[1], Wenjing Zhang[1], Chia-Yi Cheng[1], Christian Burr[1], Eric Schaller[1]**
*[1]Department of Biology, University of North Carolina, Chapel Hill, USA*

E-mail of presenting author: jkieber@bio.unc.edu

Cytokinins are $N^6$-substituted adenine derivatives that have been implicated a wide variety of plant growth and development processes. A basic framework for cytokinin signal transduction has emerged that is similar to two-component phosphorelays, which rely on the transfer of phosphates between alternating histidine and aspartic acid residues. Cytokinins are perceived by a family of histidine kinase receptors (AHKs), which, following binding of cytokinin, transfer a phosphoryl group to the histidine phosphotransfer proteins (AHPs), which in turn donate the phosphate to the response regulators proteins (ARRs) thereby regulating their activity. The ARRs fall into two groups, the type-A and type-B ARRs, which act as negative and positive elements in cytokinin signaling respectively. Two-component elements are partially functionally redundant in mediating the response to cytokinin and in various roles in regulating plant growth and development. We continue to characterize the mechanism underlying cytokinin perception and signaling, identifying an ubiquitin ligase involved in the turnover of type-B ARRs and other elements involved in cytokinin responsiveness. We are exploring how this two-component signaling pathway outputs to the many processes regulated by cytokinin. One approach that we have taken is to define and characterize the cytokinin-regulated transcriptional network. These studies include defining cytokinin responsive genes using microarray and RNA-seq approaches as well as a meta-analysis of cytokinin-regulated transcriptome data. Further, we have identified cis-acting targets to which the type-B ARRs bind *in vitro* and *in vivo*. We have also identified additional outputs of the two-component pathway by identifying proteins that interact with the type-A ARRs using yeast two-hybrid screens, and have characterized several of these interacting proteins. We continue to explore the roles of cytokinin two-component signaling elements in plant growth and development, including roles in the development of the female gametophyte and in root growth. Finally, we have begun to explore the role of cytokinin in monocots, focusing primarily on rice. Our preliminary studies include defining the cytokinin-responsive transcriptome in rice roots and shoots, and isolating rice lines altered in the function of various two-component signaling elements to define their *in vivo* roles.

# SESSION 1: BIOSYNTHESIS AND METABOLISM

## O1-1 Regulation of cytokinin biosynthesis and activity in response to nutritional cues to optimize growth and development

**Hitoshi Sakakibara[1], Takatoshi Kiba[1], Mikiko Kojima[1]**
*[1]Center for Sustainable Resource Science, RIKEN, Yokohama, Japan*

E-mail of presenting author: sakaki@riken.jp

Cytokinin (CK) plays an important role in regulation of plant growth and development, and its action is finely controlled by various steps including biosynthesis and metabolism, transport, and signaling. Previous studies have shown that *IPTs*, *CYP735As*, and *LOGs* are expressed in various parts during growth and development, and differentially regulate the synthesis of $N^6$-($\Delta^2$-isopentenyl)adenine (iP) and *trans*-zeatin (tZ). Detailed studies on *CYP735As* mutants show that tZ is important for the normal growth of shoot rather than that of root, suggesting a mechanism that modulates physiological function of CKs by modification of the side-chain structures.

As for regulation of CK action by environmental cues, it is shown that CK is a signal molecule propagating N signals throughout the whole plant body for linking N nutrition status to growth regulation, and previous studies have shown that tZ accumulation level is enhanced in response to N availability in various plants. Our recent studies show that there is dual regulation system of *AtIPT3* expression in response to nitrate ion and glutamine metabolism, as external and internal N environmental cues, suggesting that plants possess multiple regulation systems to modulate growth in response to N availability.

On the other hand, it is known that higher $CO_2$ environment enhances plant growth and development, but the role of phytohormones in the growth regulation is poorly understood. Our recent studies revealed that specific CK biosynthesis genes including *CYP735A* are up-regulated in response to higher $CO_2$ condition, and sugar derived from photosynthesis is a key signal of the regulation.

We will outline our recent progress in CK biosynthesis and its regulation in response to N and C availability, and discuss the physiological significance of regulation of CK action to optimize growth and development at whole plant level.

## O1-2 Targeted metabolomics in auxin research

**Jerry Cohen[1], Peng Yu[1], Molly Kreiser[1], Jing Chen[1], Adrian Hegeman[1]**
*[1]Department of Horticultural Science and the Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN, USA*

E-mail of presenting author: cohen047@umn.edu

Three major limiting factors in plant metabolomics are: 1) the absence of effective methods for measuring spatial distributions of metabolites, 2) a need to track multiple metabolic intermediates with high sensitivity in a single sample, 3) a lack of effective stable isotope strategies for metabolic flux analysis where metabolic steady-state assumptions often do not hold true. We have developed high-throughput mass spectrometry methods to identify and quantify plant endogenous indolic compounds in minute tissue samples. The protocols are varied based on the data required, but in general use stable-labeled internal standards and selected reaction monitoring for quantification. For unknown metabolite identification, isotopic enrichment studies, and metabolic flux analysis we have developed a very simple and sensitive analytical method that targets a wide range of indolic compounds related to IAA metabolism by high resolution LC-MS/MS on a hybrid quadrupole Orbitrap instrument by means of the highly selective quinolinium signature ion (m/z = 130.0651). The method requires minute amounts of plant material and involves no sample enrichment or purification. Finally, we have developed a series of labeling systems ranging from growth of plants on $^{13}CO_2$ for whole plant labeling (13carbon.com) and flux analysis to specific labeling methods using novel metabolic labels. Together, we are moving toward a quantitative evaluation of the function of multiple auxin-related metabolic pathways, as well as relative quantification and metabolic flux changes in core metabolic activities elicited by changes in auxin dynamics.

## O1-3 Auxin degradation – an important mechanism regulating auxin homeostasis in the Arabidopsis root

**Karin Ljung[1], Aleš Pěnčík[1,3], Biljana Simonovik[1], Sara V Petersson[1], Eva Henyková[1,3], Sibu Simon[4], Kathleen Greenham[5], Yi Zhang[5], Mariusz Kowalczyk[1], Silvana Porco[6], Malcolm Bennett[6], Mark Estelle[5], Eva Zažímalová[4], Ondrej Novák[1,3], Göran Sandberg[2]**
*[1]Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden; [2]Umeå Plant Science Centre, Umeå University, Umeå, Sweden; [3]Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, Czech Republic; [4] Institute of Experimental Botany, ASCR, Prague, Czech Republic; [5] Section of Cell and Developmental Biology, University of California San Diego, USA; [6] Plant Sciences, University of Nottingham, Nottingham, UK*

E-mail of presenting author: karin.ljung@slu.se

Auxin is a major regulator of plant growth and development, and its non-uniform distribution between cells and tissues underlies spatiotemporal coordination of many developmental events and responses to environmental stimuli. Control of auxin gradients and the formation of auxin maxima/minima most likely involve regulation of both metabolic and transport processes. We have showed that 2-oxindole-3-acetic acid (oxIAA) is a major primary IAA catabolite formed in *Arabidopsis thaliana* root and shoot tissues. The compound was highly abundant in IAA overproducing mutant lines, such as *sur1-3* and *sur2-1*, and was rapidly formed after feeding *Arabidopsis* wild type seedlings with exogenous IAA. OxIAA had very little biological activity, as shown in root and hypocotyl elongation studies, although interesting differences were observed in the binding affinity of oxIAA to different TIR1-IAA co-receptor complexes in pull-down assays.
OxIAA was the major IAA degradation product in the *Arabidopsis* root apex, and we could show that there is cell-type specific regulation of oxIAA levels in the *Arabidopsis* root apex, using cell sorting in combination with mass spectrometry analysis. OxIAA had a very low activity in *DR5:GFP* reporter assays, and transport studies showed that it is not transported via the auxin influx and efflux carriers. Our data suggest that oxIAA is an important element in the regulation of output from auxin gradients and therefore in the regulation of auxin homeostasis and response mechanisms in the root.
We recently identified two genes in *Arabidopsis* that are likely candidates for the enzymes catalysing the oxidation of IAA to oxIAA. IAA metabolite profiling of mutant and overexpressing lines in these genes showed that there are strong homeostatic mechanisms operating to regulate the auxin levels, and we are now trying to get a better understanding of these mechanisms, and how important they are for developmental processes in the root.

## O1-4 High-resolution cell-specific analysis of cytokinins in the Arabidopsis root apex

**Ondrej Novak[1], Lenka Plackova[2], Ioanna Antoniadi[3], Biljana Simonovik[4], Karel Dolezal[2], Colin Turnbull[3], Karin Ljung[4]**
*[1]Laboratory of Growth Regulators, IEB ASCR & UP Olomouc, Olomouc, Czech Republic; [2]Centre of the Region Haná for Biotechnological and Agricultural Research, UP Olomouc, Czech Republic; [3]Department of Life Sciences, Imperial College London, London, UK; [4]Umeå Plant Science Centre, Dept. of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, Sweden*

E-mail of presenting author: ondrej.novak@upol.cz
E-mail of corresponding author: karin.ljung@slu.se

Cytokinins (CKs) play crucial roles in the control of various physiological processes. Whilst metabolism provides the energy and the building blocks for plant growth and development, CKs are essential to control the rate of growth of individual plant parts (cells, tissues and organs) and to integrate the activities of these parts. High-resolution measurements of CKs in plant tissues are therefore necessary for physiological studies of their metabolism and mode of action.
Here, we applied fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with a highly sensitive mass spectrometry (MS) method for analysis of CK biosynthesis and homeostasis at cellular resolution. We modified the pipette tip solid-phase extraction (in-tip microSPE) procedure (Svačinová et al., *Plant Methods* 8:17, 2012) and applied the method to cytokinin metabolite profiling in the root tip. To confirm that the procedures of protoplast isolation and cell sorting did not alter the endogenous cytokinin levels, several control experiments were conducted. Together with the development of more sensitive and accurate MS-based methods, cell-specific analyses have provided the opportunity for CK detection in four different Arabidopsis lines, expressing GFP in specific cell types of the root apex.
In conclusion, all the well-known CK metabolites of isoprenoid CK-types could be quantified in the GFP-expressing cells. Surprisingly, no *O*-glucoside and nucleotide CK forms were detected in the stele. Our results also reveal the presence of a CK gradient within the *Arabidopsis thaliana* root tip, with a concentration maximum in the root cap, columella, initials and QC cells.

### O1-5 A facile means for the identification of indolic compounds from plant tissues

**Peng Yu[1], Adrian Hegeman[1], Jerry Cohen[1]**
*[1]Horticultural Science, University of Minnesota, Saint Paul, USA*

E-mail of presenting author: yuxxx452@umn.edu

The bulk of the indole-3-acetic acid in plants is found in the form of conjugated molecules, yet past research on identifying these compounds has largely relied on methods that were both laborious and lacked sensitivity. Utilizing recent advances in analytical instrumentation, we have developed a simple yet powerful liquid chromatography-mass spectrometry (LC-MS) based method for the facile characterization of the small IAA conjugate profile in plants. The method employs using as the signature ion the well-known quinolinium ion (130.0651 *m/z*) generated in MS processes with high mass accuracy to query the plant extract for any potential indolic compounds, including IAA conjugates. We reinvestigated soybean for its indoles and found indole-3-acetyl-trytophan (IA-Trp) in addition to the already known indole-3-acetyl-aspartic acid (IA-Asp) and indole-3-acetyl-glutamic acid (IA-Glu) conjugates. Surprisingly, several organic acid conjugates with tryptophan were also discovered, most of which are now described for the first time in plants. Our tentative studies in *Arabidopsis* also demonstrated possibilities of novel indolic compounds in the IAA biosynthetic pathway. Our method has proven to be sensitive and versatile toward the identification of novel indolic compounds. It involves minimal sample preparation but can work in conjunction with sample enrichment techniques. This method enables quick screening of IAA conjugates in both previously characterized as well as uncharacterized species and facilitates identification of novel indolic compounds in general.

### O1-6 Arabidopsis ROCK1 transports UDP-GlcNAc/UDP-GalNAc and regulates cytokinin activity and ER protein quality control

**Tomáš Werner[1], Michael Niemann[1], Isabel Bartrina[1], Angel Ashikov[2], Henriette Weber[1], Ondřej Novák[3], Louisa Brock[1], Lukáš Spíchal[3], Hans Bakker[2], Thomas Schmülling[1]**
*[1]Institute of Biology/Applied Genetics, Freie Universität, Berlin, Germany; [2]Department of Cellular Chemistry, Hannover Medical School, Hannover, Germany;[3]Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic*

E-mail of presenting author: tower@zedat.fu-berlin.de

The formation of glycoconjugates depends on nucleotide sugars which serve as donor substrates for glycosyltransferases in the lumen of Golgi-vesicles and the endoplasmic reticulum (ER). Import of nucleotide sugars from the cytosol is an important prerequisite for these reactions and is mediated by nucleotide sugar transporters (NSTs). We identified REPRESSOR OF CYTOKININ DEFICIENCY1 (ROCK1) as an ER-localized facilitator of UDP-*N*-acetylglucosamine (UDP-GlcNAc) and UDP-*N*-acetylgalactosamine (UDP-GalNAc) transport in *Arabidopsis thaliana*. Mutant alleles of *ROCK1* suppress phenotypes inferred by a reduced concentration of the plant hormone cytokinin. This suppression is caused by the loss of activity of cytokinin-degrading enzymes, cytokinin oxidases/dehydrogenases (CKXs). *rock1* enhances the shoot apical meristem activity and organ formation rate, demonstrating an important role of ROCK1 in regulating the cytokinin signal in the meristematic cells through modulating activity of CKX proteins. Our analysis showed that formation of complex and hybrid *N*-linked sugars on CKX1 was not affected by the lack of ROCK1-mediated supply of GlcNAc. We will discuss biochemical and genetic evidences indicating that the ROCK1 activity is an important part of the ER quality control system eliminating improperly folded proteins from the secretory pathway.

### O1-7 Auxin metabolites, more than a dead end?

**Elke Barbez[1], Chloe Beziat[1], Ondrej Novak[2], Antje Helmut[3], Melanie Grandits[4], Paul Staswick[5], Chris Oostenbrink[4], Luz Irina Calderon-Villalobos[5], Jürgen Kleine-Vehn[1]**
*[1]Department of Applied Genetics and Cell Biology, BOKU-university, Vienna, Austria; [2]Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany AS, Olomouc, Czech Republic; [3]Departments of Molecular Signal Processing, Leibnitz Institute of Plant Biochemistry, Halle, Germany; [4]Department of Material Sciences and Process Engineering, Institute of Molecular Modeling and Simulation, Vienna, Austria; [5]Department of Agronomy & Horticulture, University of Nebraska Lincoln, Lincoln, USA; [6]Departments of Molecular Signal Processing, Leibnitz Institute of Plant Biochemistry, Halle, Germany)*

E-mail of presenting author: elke.barbez@boku.ac.at
E-mail of corresponding author: juergen.kleine-vehn@boku.ac.at

Auxin has outstanding importance for plant development and its activity is strictly regulated at the level of signaling, transport as well as metabolism. The latter includes auxin biosynthesis, degradation, as well as its conjugation to other molecules, such as sugars, amino acids, peptides and even proteins. Amino acid auxin conjugates are so far best described and assumed to serve as auxin storage molecules or intermediate products of the auxin degradation pathway (reviewed in Lüdwig-Müller., 2011). However, it still remains to be investigated whether the diverse auxin conjugates are indeed all either inactive auxin storage molecules or degradation intermediates.
Here, we present our pharmacological as well as genetic evidences proposing an auxin metabolite to function as a signal to fine tune plant development. Our data suggests that the identified auxin metabolite may be linked to an ABP1-dependent signaling event. Transcriptomic profiling of *Arabidopsis thaliana* seedling roots revealed that auxin and the respective auxin metabolite have distinct genomic effects.
Overall, our data suggest that auxin signaling might require the integration of auxin- and auxin metabolite-dependent signaling pathways.

### O1-8 Identification of biosynthesis genes required for maintaining cytokinin homeostasis during legume nodule development

**Dugald Reid[1], Anne Heckmann[1] Ondřej Novák[2], Jens Stougaard[3]**
*[1]Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark;[2]Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic; [3]Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*

E-mail of presenting author: dugald@mb.au.dk

Symbiotic nodule development in legumes requires the reinitiation of cell divisions and establishment of a new root lateral organ. It is well established that cytokinins are required for nodule development, and cytokinin response as measured by the TCS marker has been shown locally at sites of infection and in developing nodules. Additionally, a gain-of-function variant of the *Lotus japonicus* cytokinin receptor LHK1 is sufficient to trigger spontaneous nodule development. To date little is known concerning the biosynthesis of cytokinin which leads to this activity. To address this, we searched publicly available affymetrix data for cytokinin biosynthesis genes and identified two IPT and one CKX gene with enhanced expression in the nodulated root. We subsequently identified insertion mutants in these biosynthesis genes in *L. japonicus*. Mutant analysis indicated abberant nodulation in each of these mutants and supports a role for IPT3/4 and CKX3 in maintaining cytokinin homeostasis during nodulation. Detailed expression analysis was conducted using promoter-GUS and YFP fusions. This revealed that the while the CKX3 promoter responds to Nod Factor dependent signaling and is active during the first cortical cell divisions of the nodule primordium, IPT3 and IPT4 promoter activity was not detected prior to initiation of cell divisions but was enhanced together with CKX3 later in the dividing cells of more mature nodules. Together, our analysis indicates IPT activity is required for maintaining sufficient steady-state cytokinin levels to support nodule growth but activation of existing CK pools by alternative mechanisms is likely responsible or largely responsible for the inoculation dependent accumulation of CK and resultant TCS and CKX response that rapidly follows nod-factor perception.

## O1-9 Seedy Auxins with Responsible Roles

**John Ross[1], Sandra Davidson[1], Laura Quittenden[1], Amelia Beckett[1], Sam Cook[1], Erin McAdam[1], Marion Dalmais[2], Richard Thompson[2], Nathan Tivendale[3], Noel Davies[1]**
*[1]School of Biological Sciences, University of Tasmania, Hobart, Australia; [2]Unité de Recherche en Génomique Végétale, Evry, France; [3]Department of Horticultural Science, University of Minnesota, St Paul, USA*

E-mail of presenting author: John.Ross@utas.edu.au

Recent studies on auxin biosynthesis in our laboratory indicate that the indole-3-pyruvic acid (IPyA) pathway predominates in pea (*Pisum sativum*) seeds, producing both IAA and chlorinated IAA (4-Cl-IAA). We cloned three pea aminotransferase genes, *PsTAR1*, *PsTAR2* and *PsTAR3*, which encode enzymes that convert tryptophan to IPyA (and chlorinated tryptophan to chlorinated IPyA). A null mutation in *PsTAR1* (*tar1-1*) is of great interest because we have been unable to generate homozygous recessive plants. However, transferring pollen from a heterozygous (*TAR1 tar1-1*) plant to a WT female parent results in both heterozygous and homozygous dominant offspring, as does the reciprocal cross. This indicates that both *tar1-1* pollen and *tar1-1* ovules are viable. It appears that on heterozygous plants, *tar1-1* pollen cannot fertilise *tar1-1* ovules, and therefore that homozygous mutant embryos are not formed. This indicates that auxin biosynthesis by both pollen tubes and ovules is required for fertilisation to occur. This evidence is supported by the identical effects of a second null mutation in *PsTAR1*. A null mutation in *PsTAR2* (*tar2-1*) reduces auxin content in maturing seeds (but not at younger stages) and is associated with smaller seeds, indicating a role for auxins (especially the chlorinated auxin, 4-Cl-IAA) in the later stages of seed development. The effects of *tar2-1* on the levels of other auxins, auxin precursors and conjugates in seeds are also described. For example, *tar2-1* did not reduce the content of another endogenous auxin, phenylacetic acid (PAA), indicating that enzymes other than TAR2 catalyse PAA biosynthesis. Our evidence also indicates that chlorinated indole-3-acetaldehyde (4-Cl-IAAld) originates from chlorinated IPyA, since in *tar2-1* seeds, the level of 4-Cl-IAAld is reduced. This does not mean, however, that chlorinated IAAld is an intermediate between chlorinated IPyA and chlorinated IAA.

## O1-10 Is Lonely Guy gene really lonely or it is rather Funny Guy in fungi?

**Petr Galuszka[1], Janine Schürmann[2], Josef Vrabka[1], Paul Tudzynski[2], Ondřej Novák[1]**
*[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, Czech Republic); [2]Institute of Plant Biology and Biotechnology, Westfälische Wilhelms-Universität, Münster, Germany*

E-mail of presenting author: petr.galuszka@upol.cz

Content, biosynthesis and function of plant hormones in fungal organisms have been for a long time out of scientists´ scope of interest with exception of gibberellins. Possible role of cytokinins and auxins was suggested during fungal pathogen – plant host interaction; however their production is attributed mainly to alterations of the plant metabolism. *De novo* genome assembly analysis of phytopathogenic fungus *Claviceps purpurea* revealed existence of a gene cluster with a significant homology to plant cytokinin biosynthetic genes. *C. purpurea* is a biotrophic fungus that solely infects the ovaries of flowering grasses. It replaces the ovarian tissue and finally leads to the formation of a sclerotium instead of a caryopsis. Biochemical characterization of heterologously expressed proteins from the predicted cluster, together with series of knock-out experiments, indicates that the fungus evolved a unique mechanism for the rapid production of active isoprenoid cytokinins, which was not found in any other organism so far. Cytokinins are produced in both axenic mycelial culture and during the pathogenic development *in planta*. A physiological significance and an evolutionary relationship of fungal cytokinin production will be discussed.

### O1-11 Nucleoside N-ribohydrolases from Zea mays and Physcomitrella patens

**David Kopečný[1], Martina Kopečná[1], Hanna Blaschke[2], Radka Končitíková[1], Armelle Vigouroux[3], Solange Moréra[3], Ondřej Novák[4], Miroslav Strnad[4], Klaus von Schwartzenberg[5]**
*[1]CRH – Department of Protein Biochemistry and Proteomics, Palacký University, Olomouc, Czech Republic; [2]Biozentrum Klein Flottbek, Universität Hamburg, Hamburg, Germany; [3]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France; [4]Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Olomouc, Czech Republic; [5]Biozentrum Klein Flottbek, Universität Hamburg, Hamburg, Germany*

E-mail of presenting author: kopecny_david@yahoo.co.uk

Nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-) or nucleosidases are glycosidases that catalyze the hydrolysis of the N-glycosidic bond in nucleosides to allow recycling of the nitrogenous bases and ribose. NRHs carry four aspartate residues located at the N terminus, which are involved in catalysis and coordination of a calcium ion at the active site. The binding of ribose moiety is highly conserved but the residues interacting with nucleobase vary. We performed a characterization of the NRHs from *Physcomitrella patens* (PpNRHs) and *Zea mays* (ZmNRHs). Plant enzymes belong to a class of nonspecific NRHs, which hydrolyze inosine and uridine (IU-NRHs). We identified two subclasses of plant IU-NRHs; one preferentially targets the purine ribosides inosine and xanthosine while the other is more active towards uridine and xanthosine. Both subclasses can hydrolyze plant hormones – cytokinin ribosides. We further solved crystal structures of two purine NRHs, PpNRH1 and ZmNRH3. Structural analyses, site-directed mutagenesis experiments, and phylogenetic studies allowed us to identify the residues responsible for the observed differences in substrate specificity between the subclasses. The physiological role of the PpNRHs was studied by constructing single knockout mutants. NRH deficiency resulted in delayed bud formation and PpNRH1-deficient plants did not salvage adenosine-bound nitrogen under conditions of nitrogen shortage. NRH deficiency was accompanied by changes in the levels of purine, pyrimidine and cytokinin metabolites illustrating the importance of these enzymes in nucleoside and cytokinin metabolism.

*This work was supported by grant P501/11/1591 from the Czech Science Foundation and LO1204 from the Ministry of Education, Youth and Sports of the Czech Republic.*

## POSTERS

### P1-1 Subcellular localization of cytokinin glycosyltransferase from Arabidopsis thaliana using fluorescent labeling

**Jana Dobruskova[1], Tomas Takac[2], Maria Smehilova[1]**
*[1]Department of Molecular Biology, Centre of the Region Hana for Biotechnological and Agricultural Research, Olomouc, Czech Republic; Department of Cell Biology, Centre of the*

E-mail of presenting author: jana.dobruskova@upol.cz

Cytokinins are plant hormones playing significant role in plant development, stress handling and mediating response to external environmental conditions. Levels of cytokinins in plant tissues are strictly regulated by enzymes of its metabolism. One possibility of cytokinin inactivation is their degradation by cleavage of the $N^6$-side chain by cytokinin oxidases/dehydrogenases (CKX). Other possibility is inactivation by substitution of cytokinin, while one of this substitution mechanism is glycosylation by uridine diphosphate (UDP) glycosyltransferases (UGT) forming O-glycosides or N-glycosides using UDP-glucose or UDP-xylose as the sugar donors. Cytokinin O-glycosides represent a stable storage form of cytokinin due to the possibility of reactivation by β-glucosidases, in contrast cytokinin N-glucosides can not be deglucosylated, so N-glucosylation represents irreversible cytokinin inactivation. There were identified five cytokinin specific UGTs in *Arabidopsis thaliana* genome which could differ in location at the cell level. So far, there is very little known about their subcellular localization. For example the UGT85A1 subcellular localization was determined to be cytosolic despite the prediction for chloroplast.
UGT76C2 is one the *Arabidopsis thaliana* cytokinin N-glucosyltransferases which utilizes wide range of cytokinins as substrates. UGT76C2 plays an important role in plant cytokinin response and is one of the main isoforms responsible for degradation of exogenous cytokinins.
Aim of this study was to determine subcellular localization of N-glycosyltransferase UGT76C2 using fusion with GFP. A binary vector carrying the UGT76C2:GFP under SU promotor was transformed using *Agrobacterium rhizogenes* hairy-root transformation into tomato (*Solanum lycopersicum* L.) roots. We have determined the subcellular localization of GFP-tagged glycosyltransferase by confocal microscopy, characterized phenotype modification of transgenics tissue, verified presence of transgene in tomato genome, confirmed its transcription in RT-PCR and detected the fused protein in western blot. Finally the changes in cytokinin levels were determined.

### P1-2 Effect of light and nitrogen nutrition on cytokinin metabolism of cyanobacterium Nostoc sp. PCC 7120

**Jitka Frébortová[1], Ondřej Novák[2], Ivo Frébort[3]**
[1]*Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University in Olomouc, Olomouc, Czech Republic;* [2]*Laboratory of Growth Regulators, IEB AS CR and Palacký University in Olomouc, Olomouc, Czech Republic;* [3]*Department of Molecular Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University in Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: jitka.frebortova@upol.cz

In plants, numerous developmental processes are known to be regulated by cytokinins (and other hormones as well). Although cytokinins were shown to be important also for the growth and development of cyanobacteria by increasing the rate of transcription, nothing is really known about the function of cytokinins in cyanobacteria apart from the recently reported role in a symbiotic interaction with rice roots.
Light conditions and nitrogen nutrition status are both important environmental signals that regulate diverse growth and developmental processes in plants. Multiple hormonal pathways are often modulated by the two signals to mediate the developmental changes. Conversely, hormone levels in plants influence light responsiveness and nitrogen acquisition and assimilation. In contrast to plants, some cyanobacteria are able to use atmospheric nitrogen for nitrogen nutrition in addition to fixed forms of nitrogen, being the only oxygenic photosynthetic organisms capable of nitrogen fixation. The correlation between nitrogen status or light conditions and cytokinin metabolism in cyanobacteria has not been studied to date.
To obtain clues for possible function of cytokinins in cyanobacteria, cytokinin content in the cells of *Nostoc* sp. PCC 7120 cultivated under different light regimes in nitrogen-free medium and a medium containing nitrate as a source of nitrogen was determined. Observed variations in concentrations of cytokinin metabolites will be discussed.

### P1-3 Which came first, the chicken or the egg? Is chlorosis of *atipt9* mutant caused by missing tRNA modification or just a collateral effect of iron deficiency?

**Silvia Gajdošová[1], Milada Čiamporová[2]**
[1]*Department of Botany, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University in Košice, Košice, Slovakia;* [2]*Department of Plant Physiology, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia*

E-mail of presenting author: silvia.gajdosova1@upjs.sk

One route of cytokinin biosynthesis encompasses prenylation of A37 in tRNA. The step is catalyzed by tRNA isopentenyltransferases, AtIPT2 and ATIPT9 in *Arabidopsis*, and yields isopentenyl adenine as well as *cis*-zeatin (*cis*Z). Monooxygenase, which would catalyze hydroxylation step forming *cis*Z, has not been identified in *Arabidopsis* yet. Bacterium *Salmonella typhimurium* lacking the monoxygenase is unable to grow on intermediates of Krebs cycle (fumarate, malate and succinate) unless chelated iron is supplied and a role of *cis*Z formation in regulation of iron metabolism under aerobic conditions was proposed.
Insertional mutant *atipt9* exhibits retarded growth and plant develops chlorotic true leaves. AtIPT9 can be targeted to the plastid or to the mitochondrion according to *in silico* analyses. Therefore *atipt9* phenotypic traits might result from defects related to alterations in these organelles. Chlorosis is also a common sign of iron deficiency in plants. Thus *atipt9* was cultivated on media containing iron chelate to test whether its phenotype is a consequence of decreased iron availability leading to lower chlorophyll content. Surprisingly, treatment with Fe-EDTA did not revert either chlorosis nor growth retardation. Iron uptake does not seem to be affected in *atipt9* because seedlings showed pronounced growth inhibition in the presence of iron chelate excess. Consequently, ultrastructure of mutant leaves was inspected and possible causes of detected lowered chlorophyll accumulation will be discussed.
*This research was supported by the University of P. J. Šafárik in Košice VVGS-2013-116 and VEGA1/0046/14.*

### P1-4 Nucleotide pyrophosphatase/phosphonuclease possesses the zeatin cis-trans isomerase activity in vitro

**Tomáš Hluska[1], Michaela Baková[2], René Lenobel[1], Marek Šebela[2], Petr Galuszka[2]**
*[1]Department of Molecular Biology, Centre of the Region Haná, Olomouc, Czech Republic; [2]Department of Protein Biochemistry and Proteomics, Centre of the Region Haná, Olomouc, Czech Republic*

E-mail of presenting author: tomas.hluska@upol.cz

Metabolism of isoprenoid cytokinins has been extensively studied in the past years. However, biosynthesis of *cis*-zeatin remains unknown. Besides isopentenylation of adenine in tRNA there is an another option – *cis-trans*-isomeration. In 1993, a zeatin *cis-trans* isomerase was described, purified to near homogeneity and partly characterised. However, the protein nor gene sequences were not identified yet. 20 years later, we have purified the enzyme using several chromatographic columns and gel chromatofocusing. The protein was identified as putative nucleotide pyrophosphatase/phosphonuclease. After heterologous expression we have confirmed FAD hydrolase (nucleotide pyrophosphatase) and zeatin *cis-trans* isomerase activities of the enzyme *in vitro*. Nevertheless its contribution to cytokinin metabolism *in planta* remains to be elucidated.

### P1-5 Characterization of cytokinin metabolism kinetics in Arabidopsis through experimental and computational techniques

**Petr Hošek[1], Klára Hoyerová[2], Nagavalli S. Kiran[3] Petre I. Dobrev[2]**
*[1]Department of Biomedical Informatics, Faculty of Biomedical Engineering, Czech Technical University in Prague, Kladno, Czech Republic; [2]Laboratory of Hormonal Regulation in Plants, Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Prague, Czech Republic; [3]Department of Molecular Biology and Radiobiology, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic*

E-mail of presenting author: petr.hosek@fbmi.cvut.cz

Cytokinin metabolism is rather complex network of reactions determining the concentration of particular cytokinin forms in plant tissues, thus modulating their biological effects. Kinetics of several enzymes involved in cytokinin metabolism have previously been investigated in vitro but transition of these results into physiological conditions in plants is not straightforward. Experimental studies in vivo are, therefore, still beneficial for getting an image of cytokinin metabolic machinery. Moreover, through the use of computational modelling the experimental results can be processed and kinetics of principal metabolic pathways can be estimated. In our study 14-day-old Arabidopsis seedlings were incubated in presence of trans-zeatin, cis-zeatin, dihydrozeatin and isopentenyladenin for 15, 30, 60 and 120 minutes and resulting cytokinin levels in roots and shoots were measured by HPLC. After interactive visualisation and analysis of the data the metabolic system was divided into four subsystems according to particular incubations and independent multicompartment mathematical models of these subsystems were then constructed. Multiple Monte Carlo optimizations of the models were carried out providing estimates of kinetic parameters of major reactions. Subsequent sensitivity analysis and statistical analysis provided further insight into parameter importance and reliability of the estimates.

## P1-6 Isolation and quantification of cytokinins in explants of hemp (Cannabis sativa L.) using UPLC – MS/MS

**Jakub Hrdlička[1], Iva Smýkalová[2], Magdalena Cvečková[2], Lenka Plačková[1], Ondřej Novák[3], Miroslav Griga[2], Karel Doležal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University and Institute of Experimental Botany ASCR, Šlechtitelů 11, Olomouc, Czech Republic; [2]Plant Biotechnology Department, AGRITEC Plant Research Ltd., Zemědělská 2520/16, Šumperk, Czech Republic; [3]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Faculty of Science, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic*

E-mail of presenting author: jakub.hrdlicka@centrum.cz

None of the attempts at regeneration of hemp from callus or suspension cultures by indirect organogenesis have been successful so far. Callus and suspension cultures showed tendency to form meristematic centers, however shoot formation was not observed. We aimed – with the isolation and quantification of cytokinins (CKs) and their metabolites in primary explants of *Cannabis sativa* – to better understand the actual phytohormonal situation in these explants as a background for further manipulation resulting in successful regeneration protocol. The purpose of the analysis was to determine the influence of exogenously added cytokinins on metabolism of endogenous cytokinins and generation inactive (toxic) metabolites. Samples were extracted in Bieleski buffer. A mixture of 45 deuterium-labelled cytokinin standards was added to each sample. After extraction, the supernatants were purified using tandem DEAE-Sephadex (1.0 x 5.0 cm)-octadecylsilica (0.5 x 1.5 cm) columns and an immunoaffinity chromatography (IAC) (Faiss et al. 1997). Samples were analyzed using an ultra-high performance liquid chromatography (UHPLC) (Acquity UPLC™; Waters, Milford, MA, USA) coupled to a Xevo TQ™ (Waters, Milford, MA, USA) triple quadrupole mass spectrometer equipped with an electrospray interface [ESI(+)] (Novák et al., 2008). Endogenous CK quantification was achieved by multiple reaction monitoring (MRM) of $[M + H]^+$ and the appropriate product ion. The ratio of endogenous CK to appropriate labelled standard was determined and subsequently used to quantify the level of endogenous CKs in the original hemp extract, based on the known concentration of internal standard added(Novák et al., 2008). The lowest concentration of cytokinin glucosides, especially BAP9G has been observed in plants growing on medium supplemented with BAP9THP. These results have been correlated with *in vitro* experiments.

Faiss M, Zalubilová J, Strnad M, Schmülling T, Plant J 12 (1997) 401-415
Novák O, Hauserová E, Amakorová P, Doležal K, Strnad M, Phytochemistry 69 (2008) 2214-2224

## P1-20 Identification and characterization of suppressors of rock1

**Rana Hussein[1], Michael Niemann[1], Korbinian Schneeberger[2], Tomáš Werner[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Berlin, Germany, [2]Department for Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany*

E-mail of presenting author: rana.hussein@fu-berlin.de
E-mail of corresponding author: tower@zedat.fu-berlin.de

The plant hormone cytokinin plays a crucial role in regulating growth and development. Recently, we have identified a novel nucleotide sugar transporter in *Arabidopsis thaliana* termed ROCK1 (REPRESSOR OF CYTOKININ DEFICIENCY1). ROCK1 has been found to mediate the transport of UDP-GlcNAc and UDP-GalNAc into the endoplasmic reticulum. ROCK1 is required for the activity of cytokinin-degrading enzymes, cytokinin oxidases/dehydrogenases (CKXs), and mutant alleles of *ROCK1* are able to restore the growth of cytokinin-deficienct plants overexpressing *CKX* genes. In order to identify the genetic context of *ROCK1* activity, we carried out a forward genetic screen for suppressors of *rock1*, in which *rock1 35S:CKX1* seeds were EMS-mutagenized and 1,500 lines were screened in the M2 generation. Six independent second-site *rio* (*rock is over*) alleles were isolated as causing a reversion of shoot phenotype back to that of *35S:CKX1* plant. We mapped the *rio1* mutation to the *ABCG14* locus, recently shown to code for an ATP-binding cassette transporter responsible for the acropetal translocation of the root-born cytokinin (Zhang et al. 2014, Nat Commun 5:3274). The function of different *rio* alleles in regulating cytokinin activity will be discussed.

## P1-8 Cytokinins in vacuoles

**Eva Jiskrová[1], Ondřej Novák[2], Stephanie Robert[3], Hana Pospíšilová[1], Ivo Frébort[4], Petr Galuzska[1]**
*[1]Dep. of Molecular biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic; [2]Laboratory of growth regulators, Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic; [3]Forest Genetics and Plant Physiology, Umea Plant Science Centre, Umea, Sweden; [4]Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

E-mail of presenting author: eva.jiskrova@upol.cz

Balanced level of hormones ensures right and proper plant growth. Cytokinins, one group of plant hormones, play an important role in plant development. They influence cell division and seed germination, delay the senescence, and control the growth of leaves and roots in synchronicity with auxins. Cytokinins also mediate the responses of the plant to environmental factors such as water stress, salinity, or changes in light conditions.

The metabolism of cytokinins in plant cell is effectively regulated by few enzymes and it is possible to divide them into several groups: 1. enzymes involved in biosynthesis of active cytokinins; 2. Cytokinin degradation enzymes; and 3. Enzymes maintaining inactive storage forms of cytokinin. Cytokinin dehydrogenase (CKX, EC 1.5.99.12) catalyzes the irreversible degradation of cytokinins. In *Arabidopsis thaliana*, seven forms of CKX were described. It has been shown they differ in their subcellular localization, as well as in their substrate preference. Vacuoles, the plant storage organs, accumulate various metabolites, including sugars. The presence of inactive cytokinin forms with sugar moiety and several CKX isoforms in vacuoles indicates direct connection between vacuoles and cytokinin metabolism.

In this work, we used the mutant *wat1-1* of *Arabidopsis thaliana*, which carries T-DNA insertion upstream from the ATG translation start codon of tonoplast auxin transporter, to measure the level and distribution of different cytokinin forms in vacuoles compared to the wild-type plant. Further data obtained by measuring the CKX activity with specific substrates in whole leaves, protoplasts and vacuoles prove the different subcellular localization of three forms of *Arabidopsis thaliana* CKX (AtCKX1, AtCKX2, AtCKX3). Overall, our results indicate a clear involvement of vacuoles in cytokinin metabolism.

## P1-9 High Performance Liquid Chromatography-Electrochemistry-High Resolution Electrospray Ionization/Mass Spectrometry (HPLC-EC-HR ESI/MS) Characterization of Selected Cytokinins Oxidation Products

**Michal Karady[1], Ondřej Novák[1], Aleš Horna[2], Miroslav Strnad[1], Karel Doležal[1]**
*[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic; [2]RADANAL, Ltd, Pardubice, Czech Republic*

E-mail of presenting author: ydarak@seznam.cz

Electrochemistry combined with mass spectrometry represents an emerging analytical technique used to mimick phase I. metabolism of various drugs and *in vivo* occurring compounds, often displaying a capability to generate many known metabolites or new oxidation products. An on-line LC-EC-ESI/MS method had been used to investigate the oxidation of selected cytokinin derivatives, namely isopentenyladenine (iP), *trans*-zeatin (*t*Z) and *cis*-zeatin (*c*Z). This setup allowed a rapid identification and structure elucidation of the obtained products. An electrochemical oxidation of iP resulted in five major products of oxidation and dehydrogenation reactions, correlating very well with its *in vivo* metabolism. Electrochemical conversion of *t*Z revealed six products, with two dehydrogenation products corresponding to its *in vivo* occuring metabolites. *c*Z oxidation in the electrochemical cell gave rise to eight products, resembling similarity to *t*Z oxidation. All three compounds have undergone a complete turnover mainly through two oxidation reactions occurring in the electrochemical cell – dehydrogenation and a less typical putative aliphatic hydroxylation. The resulting products are in excellent correlation with their known *in vivo* metabolism and bear the opportunity for further investigation.

### P1-10 Conversion of indole-3-buytric acid to indole-3-acetic acid in hazelnut shoot tissue

**Molly Kreiser[1], Jerry Cohen[2], Don Wyse[3]**
*[1]Department of Horticultural Science, University of Minnesota, Saint Paul, MN, USA; [2]Department of Horticultural Science and Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN, USA; [3]Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN, USA*

E-mail of presenting author: mkreiser@umn.edu

Indole-3-butyric acid (IBA) is an endogenous compound that stimulates root formation in many plant species and is also the auxin most available commercially for application to promote rooting. IBA is converted to indole-3-acetic acid (IAA) by β-oxidation in the peroxisomes. This process has been observed in a number of plant species and has been shown to be critical for normal root development in response to treatment with IBA. In this study we investigated this process in hybrid hazelnuts (*Corylus americana* x *C. avellana*), in which development of adventitious roots is a major bottleneck for vegetative propagation. Using differentially stable isotope labeled IBA and IAA tracer and internal standard, respectively, and using gas chromatography coupled with selected reaction monitoring mass spectrometry, we measured IBA-derived IAA in hazelnut shoot tissue treated with stable isotope labeled IBA. Shoot segments with fresh weights of 6-10 mg converted on average 254 ± 48 fmol of [$^{13}C_8{}^{15}N_1$]IBA to [$^{13}C_8{}^{15}N_1$]IAA over a six hour period. Screening for higher levels of IBA-to-IAA conversion may facilitate selection of hazelnut genotypes which are most easily propagated, a key trait for establishment of large-scale production systems.

### P1-11 Biological activity and quantitative analysis of 2,4-dichlorophenoxyacetic acid and its metabolites in planta

**Barbora Parizkova[1], Ludek Eyer[2], Thomas Vain[3], Jana Oklestkova[1], Hana Kozubikova[4], Tomas Pospisil[4], Milan Franek[2], Miroslav Strnad[1], Stephanie Robert[3], Ondrej Novak[1]**
*[1]Laboratory of Growth Regulators, IEB ASCR & UP Olomouc, Olomouc, Czech Republic; [2]Analytical biotechnology, Veterinary Research Institute, Brno, Czech Republic; [3]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden; [4]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

E-mail of presenting author: Parizkova.barbora@seznam.cz
E-mail of corresponding author: ondrej.novak@upol.cz

2,4-dichlorophenoxyacetic acid (2,4-D) is a chlorinated phenoxy herbicide widely used in gardens and farming to control broadleaf weeds. We have prepared several structural analogues and potential metabolites of 2,4-D and their biological activity was tested on *Arabidopsis thaliana* lines. Seedlings grown in presence of 2,4-D and to a lesser extend 2,4-D glutamic acid displayed a dose-dependent inhibition of primary root development, whereas no effect of 2,4 D aspartic acid was observed. We also demonstrate that 2,4-D metabolites are acting through a nuclear auxin signalling pathways via testing their effect on auxin-signalling deficient mutants. This was confirmed by the fact that 2,4-D and its metabolites induce a significant increase of pDR5::GUS expression. Interestingly, we could show that 2,4-D aspartic acid is less potent than 2,4-D glutamic acid and they are both less active than 2,4-D itself.
Based on the detected biological activity*in vivo* and in order to know how these compounds are assimilated *in planta*, we have developed and validated an efficient method for the quantitative analysis of the 2,4-D and its potential metabolites/analogues presence in the plant matrices. Subsequently to a one-step solid-phase extraction, an immunoaffinity chromatography was used for the isolation of 2,4-D and metabolites. The process was completed by an ultra-high performance liquid chromatography-tandem mass spectrometry method to detect 8 analytes in 7.5 minutes. The quantitative analysis of 2,4-D and its metabolites in Arabidopsis confirmed their biological activity and will be presented.

## P1-12 Ultrarapid auxin metabolite profiling for high-throughput Arabidopsis mutant screening

**Aleš Pěnčík[1], Ondřej Novák[2], Veronika Pilařová[3], Ruben Casanova Saez[1], Karin Ljung[1]**
*[1]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden; [2]Laboratory of Growth Regulators & Centre of the Region Haná for Biotechnolical and Agricultural Research, Palacký University & Institute of Experimental Botany AS CR, Šlechtitelů 11, Olomouc, Czech Republic; [3]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Heyrovského 1203, Hradec Králové, Czech Republic*

E-mail of presenting author: alespencik@seznam.cz

The phytohormone auxin (Indole-3-acetic acid; IAA) has a fundamental role in plant growth and development, acting as a signal molecule in several developmental processes. Crucial for its action is the formation of local auxin gradients, which are resulting from the interplay between auxin transport, biosynthesis, degradation and conjugation. When studying pathways of auxin metabolism, it is crucial to combine data obtained from genetic investigations with the identification and quantification of individual metabolites. In such cases, a high-throughput metabolite profiling method for rapid mutant screening would be a very valuable tool.
We are presenting a new high-throughput method for simultaneous screening of IAA and its key precursors and metabolites in minute amounts (<10mg fresh weight) of *Arabidopsis thaliana* tissues. For the isolation of IAA metabolites from plant extracts, a simple one-step purification protocol based on intip microSPE (micro **S**olid-**P**hase **E**xtraction) was utilized. Combining two types of reversed phase sorbents, we achieved a more than 80% extraction recovery of all analyzed compounds. We then merged this intip microSPE technique with fast liquid chromatography during the final mass spectrometry step to facilitate the rapid analysis of a large number of samples in very short time, and we applied the method on a large collection of Arabidopsis mutant lines which were isolated based on their leaf phenotypes. Finally, multivariate data analysis was used to evaluate the large data set generated, in order to identify mutants that were altered in their metabolite profile. Together with genetic screening, the new high-throughput auxin metabolite profiling approach will provide new insights into the pathways and regulation of auxin metabolism in Arabidopsis thaliana.

## P1-13 Isolation of cytokinins from Arabidopsis thaliana by immunoaffinity purification on magnetic micro- and nanoparticles

**Lenka Plačková[1], Jana Oklešťková[2], Kristýna Pospíšková[3], Kateřina Poláková[4], Ondřej Novák[2], Karel Doležal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of Region Haná for Biotechnological and Agricultural Research, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic; [2]Laboratory of Growth Regulators & Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University & Institute of Experimental Botany AS CR, Šlechtitelů 11, Olomouc, Czech Republic; [3]Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic; [4]Regional Centre of Advanced Technologies and Materials, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic*

E-mail of presenting author: Leninek.p@seznam.cz

The plant hormones cytokinins (CKs) are active at low concentrations ($10^{-10}$ – $10^{-15}$ mol/g FW) and their purification requires a combination of appropriate SPE separation and imunoaffinity chromatography with bonded antibodies. We have developed a new miniaturized method for immunoaffinity purification of cytokinins for selective and effective sample cleaning process. Immunoaffinity extraction as a final pre-treatment step, which produces highly purified cytokinin metabolites, is based on group-specific monoclonal anticytokinin antibodies immobilized to amagnetic micro- and nanoparticles. The antibodies were specifically bound to the free amino group of modifiers chitosan-coated magnetic microparticles (partical size 20 – 100 μm) and to the free carbonyl group of magnetic nanoparticles (synthetic and bacterial $Fe_3O_4$, particle size 20-50 nm). Firstly, the purification protocol of method was tested using samples containing only various concentration levels (0.1; 0.5 and 1 pmol) of cytokinins standards. The validation of purification process was performed using extracts of 5 mg fresh weight of 10-day-old *Arabidopsis thaliana* plants spiked with mixture of 10 fmol; 100 fmol and/or 1000 fmol of cytokinins standards. The application of magnetic microparticles was used for shoots of *Arabidopsis thaliana* (50 pcs/sample and/or 100 pcs/sample) and whole plants (1 mg; 5 mg; 10 mg and/or 20 mg). Finally, the process was completed by UPLC-MS/MS ultra-fast analysis of wide range of naturally occurring cytokinins (bases, ribosides and *N9*-glucosides) in 3.5 minutes. The efficiency of extraction and purification procedure was found to be in range of 30 – 80%, depending on individual cytokinins. The immunoaffinity purification on magnetic micro- and nanoparticles is fast, easy and effective procedure that leads to a reduction of solvent consumption.

### P1-14 Polyspecific monoclonal antibody-based immunoaffinity purification of auxins

**Jakub Rolčík[1], Luděk Eyer[2], Jana Oklešťková[1], Miroslav Strnad[1]**
*[1]Laboratory of Growth Regulators, Palacky University in Olomouc, Olomouc, Czech Republic; [2]Veterinary Research Institute, Brno, Czech Republic*

E-mail of presenting author: jakub.rolcik@seznam.cz

Current analytical chemistry of phytohormones requires highly sensitive instrumentation allowing quantification at femtomolar level or even lower. However, the preceding analytical procedure intended to remove from complex plant matrix ballast compounds might be substantially empowered by including immunoaffinity purification based on a specific interaction of antibodies with the analyte (phytohormone).
In our research, we raised a polyspecific monoclonal antibody interacting with auxin (IAA) and its amino acid conjugates, namely with Asp and Glu, which are understood to be unhydrolyzable products of IAA degradation. We developed an analytical protocol combining specific immunoaffinity purification and sensitive UHPLC-MS/MS instrumentation and used it to analyze IAA and its amino acid conjugates in Arabidopsis root tips of various genotypes.

### P1-15 Cytokinin profiling in Cyanobacteria and microalgae species using UHPLC-MS/MS

**Jan Šimura[1], Ondřej Novák[1], Miroslav Strnad[1], Ladislav Nedbal[2]**
*[1]Laboratory of Growth Regulators, Šlechtitelů 21, 783 71 Olomouc, Czech Republic, Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic; [2]Global Change Research Centre ASCR, Drasov 470, 664 24 Drasov, Czech Republic, Global Change Research Centre ASCR, Nové Hrady, Czech Republic*

E-mail of presenting author: jan.simura@gmail.com

For cytokinin biosynthesis two different pathways have been described: 1*) de novo* biosynthesis of free cytokinins and 2) the liberation of cytokinins from *t*RNA. In present work we have determined endogenous levels of the free cytokinins and *t*RNA-bound cytokinins, both were quantified in several representatives of microalgae and Cyanobacteria over phylogenetic tree. A new method for extraction and purification of *t*RNA-bound cytokinins was developed based on phenol/m-cresol treatment followed by alkaline hydrolysis and enzymatic dephosphorylation of nucleotides. Samples were purified using MCX SPE column with a cation exchange properties and then analyzed on UHPLC-MS/MS. Samples for the free cytokinins analysis were prepared using two ion-exchange chromatography (IEC) steps (SCX, DEAE-Sephadex combined with SPE C18-cartridges) and immunoaffinity chromatography (IAC). The predominant free cytokinins present in dry samples were isopentenyle-type (iP) in Cyanobacteria and mostly *c*Z-type (*cis*-zeatin) in microalgae. Four cytokinins [*cis*-zeatin ribosides (*c*ZR), $N^6$-(2-isopentenyl) adenosine ribosides (iPR), *trans*-zeatin ribosides (*t*ZR), dihydrozeatin ribosides (DHZR)] were detected in the *t*RNA extracts and these generally occurred in higher concentrations compared to the free cytokinin forms. In microalgae, the *c*Z-type cytokinins were more abundant form of *t*RNA-bound cytokinins, whereas iP-type cytokinins were mostly prevalent in Cyanobacteria. Moreover all of the tested Cyanobacteria and microalgae species showed distinct correlation between ratios of *t*RNA-bound CKs types and free CKs types contained in the same species. These results are fully compatible with the assumption that *t*RNA-mediated cytokinin biosynthesis can be an important source of cytokinins. And finding that *c*Z-type and iP-type prevalence differs among these species, shows that there are differences in cytokinin metabolism between Cyanobacteria and microalgae.

## P1-16 Aromatic cytokinins: biosynthesis and perception

**Petr Tarkowski[1], Pavel Jaworek[1], Kateřina Podlešáková[2], David Kopečný[1]**
*[1]CRH – Department of Protein Biochemistry and Proteomics, Palacky University, Olomouc, Czech Republic; [2]Department of Biochemistry, Palacky University, Olomouc, Czech Republic*

E-mail of presenting author: petr.tarkowski@upol.cz

Cytokinins (CKs) are a group of plant hormones that play a part in various biological processes in plants, such as cell division, cell differentiation, apical dominance, shoot development and leaf senescence. Up to date, occurrence, function, biosynthesis and metabolism, perception and signal transduction of isoprenoid cytokinins is quite well known, while our knowledge of aromatic cytokinins (ARCKs) remains very limited. We performed screening for ARCKs in 13 *populus* species by UHPLC-MS/MS. Results show that *Populus x canadensis Moench (cv Robusta)* seems to be the best plant model for ARCK biosynthesis studies. For such purposes, poplar cell suspension culture has been derived and characterized. Mass spectrometric data show that cell production of phenolics and aromatic cytokinins drops by 60% in 14 months of cultivation (t-zeatin/NAA). In addition, we identified *o*-topolin moiety in tRNA isolated from mature poplar leaves. While the endogenous levels of other ARCKs show transient increase after daybreak, the levels of tRNA derived *o*-topolin remain unchanged. Finally, we analyzed binding affinity of ARCKs to CHASE domain of poplar receptors HK3 and CRE1 using homology modeling and docking approach.

## P1-17 Cytokinin biosynthesis and tRNA-modification in the moss Physcomitrella

**Klaus von Schwartzenberg[1], Ann-Cathrin Lindner[1], Maike Seifert[1], Daniel Lang[2], Ralf Reski[2], Kateřina Podlešáková[3], Ondřej Novák[4], Miroslav Strnad[4]**
*[1]Biocenter Klein Flottbek – Cell Biology and Phycology, Universität Hamburg, Hamburg, Germany; [2]Faculty of Biology – Plant Biotechnology, University of Freiburg, Freiburg, Germany; [3]Department of Biochemistry, Palacký University, Olomouc, Czech Republic; [4]Laboratory of Growth Regulators and Institute of Experimental Botany ASCR, Palacký University, Olomouc, Czech Republic*

E-mail of presenting author: klaus.von.schwartzenberg@uni-hamburg.de

As a bryophyte *Physcomitrella patens* shares the last common ancestor with seed plants about 450 MYA and is a well-established evo-devo model system. In moss developmental processes such as the induction of bud formation are strongly affected by cytokinins. Cytokinin biosynthesis is mediated by isopentenyltransferases (IPTs) which catalyze the prenylation of the adenine moiety at position $N^6$. Two functional types of IPTs depending on the adenine substrates are distinguished. The adenylate-IPTs, prenylating ATP, ADP and AMP, are limited to seed plants (and some plant pathogens), where they are responsible for the biosynthesis of the dominant part of cytokinins. However, the tRNA-IPTs use as substrate an ($A_{37}$) adenine 3´ adjacent to the codon of certain tRNAs from which cytokinins are released. tRNA-IPTs can be found in all organisms except Archaea.
In contrast to seed plants, where tRNA is generally not considered to be a significant source for active cytokinins, all seven IPTs coded in the *Physcomitrella* genome are tRNA-IPT homologs. *Physcomitrella* therefore possesses unique features to study the tRNA dependent cytokinin biosynthesis in plants. Our previous studies have already identified the PpIPT1 isoform as a functional tRNA-IPT (Yevdakova and Schwartzenberg, Planta 226, 683-695, 2007). The targeted gene knockout of ipt1 together with GFP-based localization studies revealed that the chloroplast-bound IPT1 is almost exclusively responsible for the $A_{37}$ modification of tRNA in Physcomitrella. Cytokinin profiling by UPLC-MS/MS revealed a strong reduction of *cis*-zeatin-type cytokinins in knockout plants, whereas levels *N6*-isopentenyladenine (iP)- and *trans*-zeatin-type cytokinins were increased (Lindner et al., JExpBot, doi:10.1093/jxb/eru142, in press, 2014). Our data provide evidence for an unexpected and additional tRNA-independent cytokinin biosynthetic pathway in moss. A comprehensive phylogenetic analysis points towards a diversification of tRNA-IPT- homologs in moss probably related to additional functions.
*This work was supported by Deutsche Forschungsgemeinschaft (DFG) Schw687/6*

### P1-18 Contributions of nucleoside ribohydrolases to cytokinin homeostasis – functional and structural studies on Physcomitrella and maize

**Klaus von Schwartzenberg[1], David Kopečný[2], Hanna Blaschke[1], Martina Kopečná[2], Radka Končitíková[2], Maike Seifert[1], Peggy Vlietland-Nowotny[1], Solange Moréra[3], Ondřej Novák[4], Miroslav Strnad[4]**
*[1]Biocenter Klein Flottbek – Cell Biology and Phycology, University of Hamburg, Hamburg, Germany), [2]Department of Protein Biochemistry and Proteomics, Palacký University, Olomouc, Czech Republic; [3]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France; [4]Laboratory of Growth Regulators and Institute of Experimental Botany ASCR, Palacký University, Olomouc, Czech Republic*

E-mail of presenting author: klaus.von.schwartzenberg@uni-hamburg.de

Nucleoside ribohydrolases (NRHs) are key enzymes of purine- und pyrimidine metabolism that catalyze the conversion of ribosides to the corresponding bases and ribose, thereby balancing salvage and catabolism of purines and pyrimidines. These enzymes have been well characterised in protists and yeast. In plants the activities of NRHs have been described in several species, but their exact role especially with respect to cytokinin metabolism is still unclear. Here we report on the identification and characterisation of the NRH family of the moss *Physcomitrella patens* with three members (PpNRH) and that of *Zea mays* (ZmNRH) with five members. Typical for NRH are four Asp residues located in a conserved DXDXXXDD motif at the N terminus. These aspartates are involved in catalysis and coordination of a calcium ion at the active site. Enzymatic properties of recombinant NRH proteins recovered from *E. coli* were determined and revealed that each plant possesses a purine and a pyrimidine preferring subclass. Enzyme assays further demonstrated that enzymes of both subclasses can hydrolyze cytokinin ribosides although with low activity. The crystal structures of PpNRH1 and ZmNRH3 were established. To analyse the physiological role of NRHs in Physcomitrella single knockout mutants for each of the corresponding genes were generated. Characterisation of knockout mutants revealed changes in the cytokinin profiles demonstrating an in planta contribution of NRHs in the cytokinin homeostasis. Phenotypic changes such as the delayed onset of buds underline a role of NRHs in cytokinin metabolism (Kopečná et al., Plant Physiol. 163,1568-83, 2013).

*This work was supported by grant P501/11/1591 Czech Science Foundation and LO1204 Ministry of Education, Youth and Sports of the Czech Republic and by grant No Schw687/7-1 Deutsche Forschungsgemeinschaft (DFG) Germany.*

### P1-19 Overexpression of bacterial halogenases in Arabidopsis thaliana to generate new chlorindol-3-acetic acids

**Antje Walter[1], Jutta Ludwig-Müller[1]**
*[1]Department of Biology, Institute of Botany, Dresden, Germany*

E-mail of presenting author: antje.walter@tu-dresden.de

Over the last few decades natural products became more important for many areas of medicine, pharmacology and agriculture. Many important antibiotics and anticancer agents are based on natural products. The introduction of a halogen (halogenation) into natural products can improve the bioactivity and bioavailibility. It is already known that the halogenated plant growth hormone 4-Chlorindol-3-acetic acid (e.g. synthesized in legumes) shows a higher activity than indol-3-acetic acid. The introduction of a halogen in the metabolism of medically important plants could lead to a variety of novel plant metabolic products with improved properties. A regioselective incorporation of a halogen atom (chloride or bromide) can be achieved by using flavin-dependet halogenases.

In this project three well-known flavin-dependet halogenases will be introduced and overexpressed in *Arabidopsis thaliana*. The project aim is to make an exactly metabolic profile of possible modified biosynthetic metabolics in these plants. In addition the effect of the presence of chlorinated indol-3-acetic acid in the plant on the growth of these plants will be analysed in detail.

## P1-20 Study of the complex formation between CKX and HIPP proteins

**Henriette Weber[1], Tianqi Guo[1], Jenny Engelmann[1], Tomáš Werner[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: weberhenriette@gmail.com
E-mail of corresponding author: tower@zedat.fu-berlin.de

The concentration of biologically active cytokinins in the plant is effectively downregulated through glycoconjugation and oxidative degradation catalyzed by intra- and extracellular cytokinin oxidase/dehydrogenase (CKX) enzymes. In our yeast two-hybrid studies, we identified several heavy metal-associated isoprenylated plant proteins (HIPPs) as CKX-interacting proteins and confirmed the protein-protein complex formation by co-immunoprecipitation experiments. In *Arabidopsis*, around 50 HIPP proteins can be defined by the plant-specific motif combination of one or two heavy metal-binding domains (HMA) and a C-terminal isoprenylation motif. Very little is known about the biological function of these proteins. In order to understand the molecular mechanism of the interaction between HIPP and CKX, we mapped the interacting regions within both proteins and started to determine the subcellular localization of HIPP-CKX interactions using the split-YFP approach. The phenotypic and molecular analysis of *HIPP*-overexpressing plants and *hipp* knock-out mutants revealed changes in cytokinin status and responses in both types of plants suggesting that the HIPP-CKX interaction is physiologically relevant. The possible mechanisms of how the HIPP-CKX interaction could modulate activity of CKX proteins will be discussed.

## P1-21 Characterization of the maize cytokinin dehydrogenase family

**David Zalabák[1], Petr Galuszka[1], Ondřej Plíhal[2], Patricie Johnová[1], Jitka Frébortová[3]**
*[1]Department of Molecular Biology, CRH, PřF, UP in Olomouc, Olomouc, Czech Republic; [2]Molecular Biology, CRH, PřF, UP in Olomouc, Olomouc, Czech Republic; [3]Department of Chemical Biology and Genetics, CRH, PřF, UP in Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: davidzalabak@seznam.cz

Plant hormones cytokinins play a crucial role in several growth and developmental processes such as regulation of meristematic tissue activity; delay of senescence; flower and seed development, seed germination and the uptake of nutrients and assimilate into sink organs. The cytokinin homeostasis is crucial for normal course of these processes and thus it must be finely tuned. One of the way how the cytokinin level can be controlled is its irreversible degradation. The enzyme responsible for this catabolic reaction is cytokinin dehydrogenase (CKX; EC 1.5.99.12). Presented research is aimed on cytokinin catabolism in maize (*Zea mays* L.) as a monocot model plant. The goal was to characterize the biochemical features of respective ZmCKX isoforms. For this purpose, nine out of thirteen ZmCKXs were heterologously expressed in *Escherichia coli*, purified by affinity and ion-exchange chromatography and biochemically characterized. The enzyme reaction rates, as well as substrate preference were determined. The detailed study of putatively nonfunctional isoform ZmCKX6 revealed the importance of a conserved HFG motif for enzyme function. The HFG-mutant ZmCKX6 enzyme was also prepared and characterized. The differences in biochemical features of maize and *Arabidopsis* CKX enzymes are discussed here.
Besides the biochemical characterization, the subcellular localization of ZmCKX isoforms was also investigated using protein tagging with green fluorescent protein (GFP). Stable expression of ZmCKX-GFP fusion proteins in *Arabidopsis thaliana* L*er* cell suspension cultures revealed the localization of three isoforms, ZmCKX2, ZmCKX5 and ZmCKX8, to vacuoles and confirmed ZmCKX1 to be secreted to the apoplast.

# SESSION 2: TRANSPORT

## O2-1 Aspects of post-transcriptional control of PIN auxin transport proteins

**Christian Luschnig**[1]
[1]*Department for Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences, Vienna, Austria*

E-mail of presenting author: christian.luschnig@boku.ac.at

Plants evolved an unparalleled plasticity in post-embryonic development, which to a significant extent is controlled by directional transport of the phytohormone auxin. As a consequence, inter- and intracellular auxin transport are subject to multifaceted control mechanisms. This involves a tight transcriptional and post-transcriptional regulation of PIN-type auxin transport proteins, which is a prerequisite for the translation of extrinsic signals into cellular differentiation programs via modification of auxin flow.
Our lab focuses on the analysis of post-transcriptional regulation of PIN proteins, which led to characterization of *cis*- and *trans*-acting determinants that affect auxin flow via impacting on PIN steady-state protein levels and intracellular distribution. Progress in the characterization of pathways involved will be presented.
*Supported by FWF grant P25931*

## O2-2 TWISTED DWARF1 regulates cytoskeleton bundling and dynamics

**Markus Geisler**[1]
[1]*University of Fribourg, Department of Biology, Switzerland*

E-mail of presenting author: markus.geisler@unifr.ch

The immunophilin-like FKBP42, TWISTED DWARF1 (TWD1), controls the polar transport of the plant hormone, auxin, by regulating the plasma membrane presence of several members of the ABCB/PGP family of auxin transporters. Absence of ABCB-mediated auxin efflux in *twd1* or *abcb* mutants results in overlapping phenotypes including dwarfism.
In order to understand the molecular basis of non-handed epidermal twisting of *twd1* plants, we employed a co-immunoprecipitation-MS/MS approach in order to identify novel TWD1 interacting proteins. We identified several actin isoforms that were themselves partially known to control root length and waving. Employing single-cell systems we found that *twd1* reveals previously overseen developmental defects and that actin single and double-mutant combinations phenocopy defects found in *twd1*. Quantification of auxin distribution and transport suggests that these defects are most likely caused by altered auxin transport capacities. Strikingly, actin reporter gene microscopy and VAEM/TIRFM revealed that these defects correlate with altered actin bundling and dynamics in *twd1*. Our findings contribute to our understanding of the role of the actin cytoskeleton in auxin transport and underline the versatile role of TWD1 as auxin transport regulator.

## O2-3 Arabidopsis ABCG14 is required for xylem loading of cytokinins for root-to-shoot translocation

**Takatoshi Kiba[1], Donghwi Ko[2], Joohyun Kang[2], Jiyoung Park[2], Mikiko Kojima[1], Mi Kwon[3], Anne Endler[4], Enrico Martinoia[4], Youngsook Lee[2], Hitoshi Sakakibara[1]**
*[1]Plant Productivity Systems Research Group, RIKEN CSRS, Yokohama, Japan; [2]Division of Integrative Biosciences and Biotechnology, POSTECH, Pohang, South Korea; [3] Department of Biotechnology, Korea University, Seoul, South Korea; [4] Institut für Pflanzenbiologie, Universität Zürich, Zürich, Switzerland*

E-mail of presenting author: tkiba@psc.riken.jp

Plants maximize fitness by coordinating growth and development of organs such as the shoot and the root in response to environmental changes. Cytokinins have been implicated in the coordination acting as a long-distance signal translocated via xylem and phloem. However, the mechanism of long-distance translocation of cytokinins remains to be determined.
We have identified the Arabidopsis ABC transporter AtABCG14 as a key component of root-to-shoot cytokinin translocation. The *atabcg14* T-DNA insertion mutant shows growth retardation in the shoot, which resembles cytokinin-deficient mutants. Application of exogenous cytokinin restores shoot growth, indicating that cytokinin-deficiency is the cause of the phenotype. In the mutant, cytokinin content in the shoot was reduced, while it was increased in the root. Translocation of radiolabeled *trans*-zeatin from the root to the shoot as well as cytokinin content in xylem sap was significantly decreased in the mutant. These results suggest that *AtABCG14* is required for xylem loading of cytokinins for root-to-shoot translocation. The physiological role of root-to-shoot translocated cytokinins will also be discussed based on grafting experiments between wild type, *atabcg14*, and cytokinin biosynthesis-related mutants now in progress.

## O2-4 Phosphorylation-mediated regulation of polar auxin transport: peripheral membrane proteins go nuclear

**Remko Offringa[1]**
*[1]Molecular and Developmental Genetics, Institute Biology Leiden, Leiden University, The Netherlands*

E-mail of presenting author: r.offringa@biology.leidenuniv.nl

As sessile organisms, plants have acquired a plethora of mechanisms to adapt and optimize their development and growth to changes in their environment. In this regulatory network, the plant hormone auxin, or indole-3-acetic acid (IAA), plays a central role, as it controls many aspects of plant growth and development through dynamic polar cell-to-cell transport-generated maxima and minima. The polarity of auxin transport is determined by the asymmetric subcellular distribution of the PIN1-type auxin efflux carriers. Reversible phosphorylation of these PIN proteins in their central hydrophilic loop through the antagonistic action of PINOID (PID) and related AGC3 kinases and PP2A phosphatases is sufficient to direct their proper localization during embryogenesis, tropic growth, and the initiation of flowers and floral organs. Detailed functional analysis of the AGC3 kinases shows that they act both redundantly and differentially in regulating auxin transport, and that their subcellular localization is a key aspect of their functionality. All four AGC3 kinases are peripheral membrane proteins, but they also show differential subcellular localization, in part determined by interacting proteins. Recently, evidence was obtained for a nuclear function for at least two of the AGC3 kinases. This new role corroborates the position of these kinase as central hubs in a regulatory network through which both internal and external signals mediate their effect on plant development.

## O2-5 Dynamical regulation of growth rate and cellular organisation of the Arabidopsis root meristem and elucidation of PIN mechanisms

**Klaus Palme[1,3,4,5], Thomas Blein[1], Jasmin Duerr[1], Taras Pasterna[1], Thomas Haser[1], Thorsten Schmidt[2], Kun Liu[2], Franck A. Ditengou[1], Olaf Ronneberger[2,3], Kryztina Kratzat[1], Jun Cho[1], Thomas Blidl[6], Uwe Schulte[6], Xugang Li[1], Cristina Dal Bosco[1], Alexander Dovzhenko[1], William Teale[1]**

*[1]Institute for Biology II, Molecular Plant Physiology, Faculty of Biology, Albert-Ludwigs-University Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany; [2]Institute for Computer Science, Image Analysis Lab, Albert-Ludwigs-University Freiburg, Georges-Köhler-Allee Geb. 52, D-79110 Freiburg, Germany; [3]BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University Freiburg, Signalhaus, Schänzlestr. 18, D-79104 Freiburg, Germany; [4]Freiburg Centre for Advanced Studies (FRIAS), Albert-Ludwigs-University Freiburg, Albertstr. 19, D-79104 Freiburg, Germany; [5]Center for Biological Systems Analysis (ZBSA), Albert-Ludwigs-University Freiburg, Habsburger Str. 49, D-79104 Freiburg, Germany; [6]Institute of Physiology, Albert-Ludwigs-University Freiburg, Herman-Herder-Str. 7, D-79104 Freiburg, Germany*

E-mail of presenting author: klaus.palme@biologie.uni-freiburg.de

To understand the cellular mechanisms that direct organ growth in response to internal and environmental cues, large-scale methods are needed for a quantitative analysis of cells in situ. This lack of quantitative methods has consequences; for instance, despite the relative simplicity of the Arabidopsis root, there is no current consensus on its three-dimensional organization and its response to environmental cues such as light. Using advanced imaging in combination with pattern analysis, we present here a quantitative cell geometry and growth analysis of Arabidopsis roots in 3D. We built a cell atlas of the Arabidopsis root tip, in which we automatically allocate all cells to specific layers and root zones and quantitatively measure their geometric dimensions in 3D. We show that different cell layers have different cell geometries and demonstrate that each cell layer has a characteristic cell shape that remains unmodified by the root growth rate. We show that the cell geometry is not homogenous, but varies in length along the root axis depending on cell division. We also demonstrate how the fates of root cells are determined over time in the presence or absence of light and relate the dynamic changes in growth with cellular geometry. We further isolated PIN complexes from Arabidopsis plasma membranes and identified the interacting proteins. These interactors were functionally and structurally analysed and mapped back to individual cells of the root tip. Moreover, we developed a ratiometric auxin transport assay with which analysed instantly the function of PIN proteins. Based on these findings the function of PIN proteins will be discussed.

## O2-6 A conceptual PIN-mediated auxin-transport model of grass vegetative leaf initiation and vein patterning

**Devin O'Connor[1], Ottoline Leyser[1]**

*[1]Sainsbury Laboratory, Sainsbury Laboratory Cambridge University, Cambridge, UK*

E-mail of presenting author: devin.oconnor@slcu.cam.ac.uk

Grass leaves have several features that are integral to their remarkable productivity. Stiff parallel veins provide mechanical stability to the blade, and allow for greater vein density. The leaf base encircles the meristem, closely covering the apex and thus protecting the meristem and younger organs. Parallel veins and an encircling leaf base are common in monocots, and are associated with a unique vascular anatomy in the stem, the atactostele. In the atactostele the vasculature of each successive leaf is nested circumferentially within the ring of vasculature derived from older organs. We previously identified a lineage-specific duplication in the PIN1 clade that gave rise to PIN1a and PIN1b in grasses. We showed that PIN1a and PIN1b act in concert with a previously uncharacterized PIN, Sister-of-PIN1, and that all three have qualitatively different expression and polarization patterns during organ initiation and vein patterning in *Brachypodium* (O'Connor *et al.* PLoS Comput Biol 2014). Here, examination of SoPIN1, PIN1a, and PIN1b during vegetative leaf development shows the three PINs have similar dynamics to those previously described for floral development. SoPIN1 shows convergent polarization at the sites of leaf initiation. PIN1b is expressed early in leaf initiation in a broad domain, while PIN1a is expressed later along the paths of incipient veins. Unlike floral development however, the expression domain of PIN1b expands dramatically below the earliest primordia coincident with SoPIN1 convergence around the entire meristem circumference. The result is a heterochronic shift, delaying vascular patterning by PIN1a and allowing for a larger portion of the meristem circumference to contribute to the leaf base thus creating a larger sink to accept more leaf veins. We outline a conceptual model for how PIN1a and PIN1b may have, after duplication, subfunctionalized the ancestral PIN1 function resulting in the encircling leaf base, atactostele, and parallel veins characteristic of monocots.

## O2-7 A chemical genomic approach to identify endogenous compounds implicated in plant growth regulation

**Stéphanie Robert[1], Adeline Rigal[1], Siamsa Doyle[1], Petr Klíma[2], Ondrej Novák[1], Thomas Vain[1], Karin Ljung[1], Eva Zažímalová[2]**
*[1]Forest Genetics and Plant Physiology, Swedish University of agricultural sciences (SLU), Umeå, Sweden; [2]The Academy of Sciences of the Czech Republic, Institute of Experimental Botany, Prague, Czech Republic*

E-mail of presenting author: stephanie.robert@slu.se

The chemical genomics approach uses small molecules for rapid and effective dissection of biological mechanisms and gene networks, in ways not feasible with mutation-based approaches. Endomembrane 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. By screening a small molecules library that had previously been established as targeting the endomembrane system (Drakakaki et al. 2011), we identified Endosidin 8 (ES8), which strongly affects the trafficking of the basal membrane-localized auxin transporter PIN1, while apical membrane-localized PIN2 is almost unaffected. Additionally, ES8 activity inhibits auxin transport in BY-2 cells but does not affect the total auxin level in *Arabidopis* seedlings. Structurally ES8 is an analogue of anthranilic acid (AA), a precursor of aromatic amino acids, including tryptophan (Radwanski and Last, 1995). Other identified AA-analogues significantly modify PIN1 trafficking and auxin transport similarly to ES8. The anthranilate synthase deficient double mutant *wei2-1;wei7-1* (Stepanova et al., 2005) displays a defect in gravitropic response and root development. While AA fully rescues the phenotype of *wei2-1;wei7-1,* AA-synthetic analogs recover only the gravity defect without modifying the root growth. Opposite to AA itself, AA-analogues do not influence tryptophan level suggesting that they do not modulate tryptophan-dependent auxin biosynthesis. Taken together, by means of chemical genomics, we have reveal a novel role of AA in the modulation of basally localized auxin carrier trafficking, independent of its function in auxin biosynthesis.

## O2-8 Strigolactone transport requires apical-basal and lateral localization of the ABC protein PDR1

**Lorenzo Borghi[1], Joelle Sasse[1], Sibu Simon[2], Christian Gübeli[1], Enrico Martinoia[1], Jiri Friml[2]**
*[1]Institute of Plant Biology, University of Zurich UZH, Zurich, Switzerland; [2]Developmental and Cell Biology of Plants, Institute of Science and Technology Austria IST, Klosterneuburg, Austria*

E-mail of presenting author: lorenzo.borghi@uzh.ch

Strigolactones are the most recently discovered plant hormones. In 2005 strigolactones were reported to be involved in establishing the mycorrhiza, a plant fungus interaction possibly required for land colonization (Akiyama et al., 2005). In 2008 strigolactones together with auxins were shown to determine the plant architecture by inhibiting bud outgrowth (Gomez-Roldan et al., 2008; Umehara et al., 2008). We recently published the discovery of the Petunia strigolactone transporter PaPDR1 (Kretzschmar et al., 2012). However, the mechanisms behind strigolactone allocation / exudation were not yet known.
We here show that PaPDR1 is present in the apical membrane of root-tip cortex cells, where it co-localizes with the auxin transporter PIN2, and it is outer-laterally localized in hypodermal passage cells. The evidences for PaPDR1 asymmetrical localization come from in vivo analyses and immunolocalization approaches by mean of the fusion protein GFP-PDR1 inserted in Petunia and Arabidopsis. Mutants for pdr1 do not export radiolabelled GR24, a sinthetic strigolactone, out of the root tip as efficiently as wild type. Additionally, pdr1 mutants feedback-regulate strigolactone biosynthesis. Therefore, PaPDR1 strigolactone transport plays a key role in root tip homeostasis. We also show that GFP-PDR1 vesicles are BFA sensitive, as it was previously shown for GFP-auxin-transporter protein fusions (Geldner et al., 2001). Therefore PaPDR1 might share part of the vesicle traffic mechanism which is responsible for the polar localization of PIN proteins. Together with auxins, strigolactones are so far the only phytohormone showing a polar localization of their transporter. Interestingly, auxins and strigolactones adjust their activities by respectively increasing strigolactone biosynthetic and transporter levels (Kretzschmar et al., 2012) or increasing PIN endocytic recycling, thus dampening auxin transport (Crawford et al., 2010). We propose that the polar transport of these two phytohormones might be necessary to compartmentalize their feedback actions thus to regulate plant development.

# POSTERS

## P2-1 Subcellular distribution and function of PIN proteins with reduced central hydrophilic loop from tobacco

**Mária Čarná[1], Karel Müller[1], Markéta Pařezová[1], Martina Laňková[1], Jan Petrášek[1], Eva Zažímalová[1]**
*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, v. v. i., Prague, Czech Republic*

E-mail of presenting author: carna@ueb.cas.cz
E-mail of corresponding author: zazimalova@ueb.cas.cz

Plant hormones and auxin in particular have been shown to regulate many aspects of plant development. Morphogenic action of auxin is dependent on auxin homeostasis, which is at the cellular level maintained by concerted activities of auxin biosynthesis, metabolism and transport. With respect to the cell-to-cell transport of auxin, several auxin transporters were described and their subcellular redistribution was shown to contribute to control of auxin transport across plasma membrane. In this work, we have concentrated on the role of auxin carriers with reduced central hydrophilic loop, thus addressing the relationship between their action and the overall auxin redistribution and homeostasis in tobacco cells. We have cloned 4 PIN-FORMED (PIN) proteins from the genome of *Nicotiana tabacum* L. (Nt*PIN5*, Nt*PIN6*,Nt*PIN8* and Nt*PIN10*) and overexpressed them under inducible promoters in tobacco BY-2 cells. Auxin transport assays demonstrated that *Nt*PIN6 has auxin efflux activity for both naphthalene-1-acetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). Its expression also induced the auxin-starvation phenotype of individual cells. In contrast, Nt*PIN5* overexpression did not increase efflux of 1-NAA and did not change cell morphology. Interestingly, 2,4-D efflux was slightly increased in cells overproducing *Nt*PIN5. IAA metabolic profiling was performed after the overproduction of *Nt*PIN5 and *Nt*PIN6,and their subcellular localization in HA-tagged lines was also determined. Altogether, the results suggest close relationship between the size of central hydrophilic loop of individual tobacco PIN auxin carriers and their subcellular localization and function.

*This work was supported by the Grant Agency of the Czech Republic, project GAP305/11/0797 (to EZ).*

## P2-2 A chemical genomic approach to identify new players involved in polar targeting

**Urszula Kania[1], Stephanie Robert[2], Glenn Hicks[3], Natasha Raikhel[3], Jiri Friml[1]**
*[1]Institute of Science and Technology Austria (IST Austria), Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria; [2]Department of Forest Genetics and Plant Physiology, Umea Plant Science Centre (UPSC), Swedish University of Agricultural Sciences, Umea, Sweden; [3]Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, USA*

E-mail of presenting author: urszula.kania@ist.ac.at

Establishment of cell polarity is one of the most fundamental topics in cell biology. In plants, polarity at the cellular as well as tissue level is manifested and linked by the directional transport of a plant signalling molecule auxin, which mediates a variety of plant developmental responses. The polar localization of PIN auxin transporters determine the directionality of auxin transport and contribute to the establishment of differential auxin distribution within tissues and coordinated tissue polarization. So far, only little is known about how this polar localization is established and maintained. Here we used an innovative chemical genomics approach to address mechanism of polar targeting in plants and screened libraries of small molecules for modifiers of PIN polar localization. In a first round, we performed a high-throughput screen based on the highly polarized process of pollen germination. Potential inhibitors of pollen germination were selected and tested on their effect on polar PIN localization. By this approach, we selected and characterized set of bioactive chemicals affecting basal PIN localization or PIN trafficking. We expect that these will be instrumental to identify novel regulators of vesicle trafficking and polarity in plants.

### P2-3 Arabidopsis nucleoside transporter ENT3 influences cytokinin uptake, content and synthesis

**Alla Korobova[1], Torsten Möhlmann[2], Bulat Kuluev[1], Guzel Kudoyarova[1], Stanislav Veselov[3]**
*[1]Laboratory of Plant Physiology, Institute of Biology, Ufa Research Centre, Russian Academy of Sciences., Ufa, Russian Federation; [2]Department of Plant Physiology, University of Kaiserslautern, Kaiserslautern, Germany; [3]Department of Biology, Bashkir State University, Ufa, Russian Federation*

E-mail of presenting author: muksin@mail.ru

Competitive uptake studies in yeast cells suggested that nucleoside transporters belonging to the *ENT* family are capable of cytokinin-riboside transfer across membranes (Hirose et al., 2005). However, their involvement in the control of cytokinin accumulation in plants has not been studied. We addressed this by research of Arabidopsis *ent3* mutants compared to Colambia wild type (WT) plants. The level of cytokinin accumulation in 4-week-old mutant and WT plants grown in hydroponics was compared 1 day after addition of $4 \times 10^{-7}$ M zeatin riboside (ZR) to the medium. The increase in cytokinin content due to ZR uptake was 150 and 70 pmol/g fresh weight in roots of WT and ent3 plants, correspondingly. Thus, accumulation of exogenous cytokinin was two-times lower in mutant plants confirming contribution of intact ENT3 transporter to ZR uptake. Meanwhile the content of endogenous cytokinins was about 2-3 times higher in mutant than in WT. To study if this was due to the rate of cytokinin decay we estimated activity of cytokinin oxidase as described (Vysotskaya et al., 2010) and detected no difference between genotypes. Then transcript level of genes coding for isopentenyltransferases was estimated by means of real-time PCR (actin gene serving as a reference) using the same primers as described (Miyawaki et al., 2004). We detected about 1.8-2 times higher transcript level of AtIPT3 and AtIPT5 in roots of *ent3* mutant as compared to WT. These results suggest that higher cytokinin content in the mutant was due to the increased synthesis compensating for decreased ability of the plants for cytokinin uptake. The linkage between cytokinin synthesis and transport detected in our work is in accordance with the previous data of Sun et al (2005) who identified *AtENT8* (relatedto*AtENT3*) in an effort to screen for suppressor of the mutants overproducing cytokinins.
*Supported by RFBR-No-13-04-00666.*

### P2-4 Characterization of NRT1.1-driven auxin transport activity on the cellular level

**Martin Kubeš[1], Phillippe Narcry[2], Alain Gojon[2], Eva Zažímalová[1], Klára Hoyerová[1]**
*[1]Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Rozvojová 263, 165 02 Prague 6, Czech Republic; [2]Biochimie et Physiologie Moléculaire des Plantes, CNRS/INRA/SupAgro-M/UM2, Montpellier 34060, France*

E-mail of presenting author: kubes@ueb.cas.cz
E-mail of corresponding author: Hoyerova@ueb.cas.cz

NRT1.1 performs a dual function – it acts as nitrate transporter and sensor. Besides, a unique signaling mechanism by NRT1.1 via auxin transport resulting in regulation of lateral root growth by nitrate was recently proposed. Under limited nitrate availability, the NRT1.1-based auxin transport kinetics changes so that auxin is transported from lateral roots basipetally. This results in lower auxin accumulation in lateral root tips and consequently in repression of lateral root growth. Contrary to this, under high nitrate concentration, NRT1.1 protein is blocked as an auxin carrier, basipetal auxin transport from lateral roots is suppressed and auxin level in root tips is elevated resulting in promotion of lateral root growth. However, molecular basis for functioning of NRT1.1 as an auxin transporter is not yet fully understood. Using tobacco BY-2 cell suspension culture, we have characterized substrate specificity of the NRT1.1-facilitated auxin transport, and revealed some aspects of regulation of NRT1.1 activity.

### P2-5 Role of AUXIN BINDING PROTEIN 1 (ABP1) in dynamics of plasma membrane residing proteins

**Jozef Lacek[1], Eva Zažímalová[1], Jan Petrášek[1], Milada Čovanová[1]**
*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR v.v.i., Prague, Czech Republic*

E-mail of presenting author: joseflacek9@gmail.com
E-mail of corresponding author: covanova@ueb.cas.cz

ABP1 is considered as auxin receptor; its function is important for many auxin-driven processes, including modulation of auxin homeostasis in cells by regulation of intercellular auxin flow mediated by plasma membrane (PM)-localized auxin efflux carriers. The involvement of ABP1 in regulation of clathrin-dependent endocytosis has been confirmed and shown to include not only auxin efflux carriers of PIN-type but also other PM proteins such as aquaporins from PIP-family and part of population of PM $H^+$-ATPase.
Therefore, we have examined the activity of ABP1 in regulation of dynamics of selected PM proteins known to utilize clathrin-dependent pathway, such as auxin efflux carrier PIN1, PM $H^+$-ATPase PMA4 and aquaporins PIP1 and PIP2, but also the auxin transporters ABCB4 and AUX1 that are likely to utilize other type of vesicle transport. To study the ABP1-driven protein trafficking we used methods of confocal microscopy in tobacco BY-2 cell lines co-expressing ABP1 along with selected fluorescently tagged proteins (PIN1-GFP, PMA4-GFP, PIP1,2-GFP, PIP2,1-GFP, ABCB4-GFP and AUX1-YFP). Our results show that regulation by ABP1 of PM proteins cycling is not dependent on type of vesicles that those proteins use. It is more probable that function of particular protein is more important factor in this regulatory mechanism.
*The work was supported by the grant Agency of the Czech Republic, project no.: GAP501/12/P951 (to MČ)*

### P2-6 Auxin transport across plasma membrane does not depend on the intracellular and plasma membrane dynamics of auxin influx and efflux carriers

**Martina Laňková[1], Jana Humpolíčková[2], Martin Hof[2], Eva Zažímalová[1], Jan Petrášek[1]**
*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Prague, Czech Republic; [2]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

E-mail of presenting author: lankova@ueb.cas.cz
E-mail of corresponding author: petrasek@ueb.cas.cz

A significant proportion of the morphogenic action of auxin has been shown to be regulated by carrier-mediated cell-to-cell auxin transport. Auixn carriers transport auxin across plasma membrane as well as across some endomembranes. However, the contribution of auxin-transporting activity of individual carriers within the membrane of individual endosomes to the overall balance of intercellular auxin transport is still a point of debate. Using suspension-cultured cells of tobacco and Arabidopsis we have determined the kinetics of carrier-mediated auxin influx and efflux after the treatment with cytoskeletal drugs. We show that depolymerization and stabilization of AFs did not decrease auxin influx or efflux in comparison with control. The same applies for MTs, but here slight decrease in the auxin efflux was observed after their depolymerization. Tracking of trajectories of individual endosomes with PIN1-GFP and AUX1-YFP after treatment with cytoskeletal drugs revealed their cytoskeleton-dependent movement. The speed of fluorescence recovery after photobleaching (FRAP) of heterologously expressed *Arabidopsis thaliana* PIN1-GFP and AUX1-YFP uncovered that the mobile fraction of both auxin efflux and influx carriers seems to be quite low within the plasma membrane. Moreover, FRAP of both PIN1-GFP and AUX1-YFP is not changed after upon cytoskeleton depolymerization. On the other hand, raster image correlation spectroscopy (RICS) on confocal sections through the region of plasma membrane and adjacent layer of cytoplasm revealed that this fraction of PIN1 is more dynamic then AUX1. High mobility of PIN1 detected with RICS is shown here to depend on actin and microtubular cytoskeleton in contrast to AUX1 that depends on sterols. Our results suggest that the cytoskeleton-mediated deposition of membrane vesicles and dynamics of auxin carriers within the plasma membrane do not contribute to the fine tuning of the amount of auxin being transported across plasma membrane.
*This work is supported by the Czech Science Foundation, project GAP305/11/2476.*

### P2-7 *Nicotiana tabacum* PIN auxin carriers: identification of gene family, expression profiling, localization studies and functional testing

**Karel Müller[1], Mária Čarná[1], Markéta Pařezová[1], Kateřina Malínská[1], Eva Zažímalová[1], Jan Petrášek[1],**
*[1]Lab. of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, v.v.i., Prague, Czech Republic*

E-mail of presenting author: muller@ueb.cas.cz
E-mail of corresponding author: petrasek@ueb.cas.cz

Plant cell strains isolated from *Nicotiana tabacum* plants (BY-2, VBI-0) are frequently used to track auxin transport mechanisms at the cellular level. They allow precise determination of auxin transport activities of heterologously expressed auxin transporters. However, information on the genes coding for endogenous, tobacco auxin transporters is largely missing. Here we report on the identification of PIN auxin carrier family from *Nicotiana tabacum* genome. Sequences from both genomic DNA and cDNA from *Nicotiana tabacum*, its ancestors *Nicotiana sylvestris* and *Nicotiana tomentosiformis* and closely related *Nicotiana benthamiana* allowed us to identify and clone 9 Nt*PIN* genes. Some of them (Nt*PIN1, 2, 3, 4*) were found in very closely homologous pairs suggesting their origin from two ancestral genomes. To analyze the role of individual Nt*PINs* we measured transcript levels by qRT-PCR in various plant tissues and during BY-2 cell culture growth and cell cycle. The results indicate tissue-specific expression in plants and growth phase-specific expression in cell culture. Here, Nt*PIN2* was found to be specific for exponential growth phase, while Nt*PIN3* and Nt*PIN4* showed higher expression during cell elongation and stationary phase. In contrast, the expression of all PINs was not cell cycle stage-specific. Inducible expression of individual members of *NtPIN* family in tobacco BY-2 cells was performed to determine the auxin-transporting activity of individual members of tobacco PIN family and to follow their impact on the overall cellular morphogenesis. Results suggest that plasma membrane *Nt*PINs differ in their capacities to transport synthetic auxins 1-Naphthaleneacetic acid and 2,4-Dichlorophenoxyacetic acid.
Altogether, our results suggest complex regulation of individual members of tobacco PIN family in the homogeneous population of undifferentiated cells.
*This work was supported by the Czech Science Foundation, projects GAP305/11/2476 (to JP) and GAP305/11/0797 (to EZ).*

### P2-8 Enquiry into the topology of plasma membrane localized PINs

**Tomasz Nodzyński[1], Steffen Vanneste[2], Jiří Friml[3]**
*[1]Mendel Centre for Plant Genomics and Proteomics, Central European Institute of Technology (CEITEC), Masaryk University (MU), Brno, Czech Republic; [2]Department of Plant Systems Biology, Ghent University, VIB, Ghent, Belgium; [3]Developmental and Cell Biology of Plants, Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria*

E-mail of presenting author: tomasz.nodzynski@ceitec.muni.cz
E-mail of corresponding author: jiri.FRIML@ist.ac.at

A significant amount of experimental data has been published in scientific literature concerning the importance of auxin guiding plant ontogenesis. This phytohormone accumulates differentially in plant tissues coordinating the onset of various developmental stages. The formation of local auxin maxima is achieved largely by PIN proteins among which, PINs 1, 2, 3, 4 and 7 localize asymmetrically at the PM of cells facilitating the directional cell to cell transport of auxin. The developmental importance of subcellular trafficking and localization of PINs has been intensively studied however, little is known about the structure of those efflux carriers. Here we present experimental data concerning the topology of plasma membrane localized PINs. We utilize and reconcile multiple software predictions in order guide the experimental topology determination process. Utilizing PIN versions fussed with fluorescent reporters or epitope tags and employing a modified immunolocalization protocol we map the membrane topology of those auxin efflux carriers.

### P2-9 A chemical genomic approach to identify endogenous compounds implicated in plant growth regulation

**Adeline Rigal[1], Siamsa Doyle[1], Petr Klíma[2], Ondrej Novák[1], Thomas Vain[1], Karin Ljung[1], Eva Zažímalová[2], Stéphanie Robert[1]**
*[1]Forest Genetics and Plant Physiology, Swedish University of agricultural sciences (SLU), Umeå, Sweden; [2]The Academy of Sciences of the Czech Republic, Institute of Experimental Botany, Prague, Czech Republic*

E-mail of presenting author: adeline.rigal@slu.se
E-mail of corresponding author: stephanie.robert@slu.se

The chemical genomics approach uses small molecules for rapid and effective dissection of biological mechanisms and gene networks, in ways not feasible with mutation-based approaches. Endomembrane 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. By screening a small molecules library that had previously been established as targeting the endomembrane system (Drakakaki et al. 2011), we identified Endosidin 8 (ES8), which strongly affects the trafficking of the basal membrane-localized auxin transporter PIN1, while apical membrane-localized PIN2 is almost unaffected. Additionally, ES8 activity inhibits auxin transport in BY-2 cells but does not affect the total auxin level in *Arabidopis* seedlings. Structurally ES8 is an analogue of anthranilic acid (AA), a precursor of aromatic amino acids, including tryptophan (Radwanski and Last, 1995). Other identified AA-analogues significantly modify PIN1 trafficking and auxin transport similarly to ES8. The anthranilate synthase deficient double mutant *wei2-1;wei7-1* (Stepanova et al., 2005) displays a defect in gravitropic response and root development. While AA fully rescues the phenotype of *wei2-1;wei7-1,* AA-synthetic analogs recover only the gravity defect without modifying the root growth. Opposite to AA itself, AA-analogues do not influence tryptophan level suggesting that they do not modulate tryptophan-dependent auxin biosynthesis. Taken together, by means of chemical genomics, we have reveal a novel role of AA in the modulation of basally localized auxin carrier trafficking, independent of its function in auxin biosynthesis.

### P2-10 Auxin in freshwater green algae

**Roman Skokan[1], Petr Skůpa[1], Jan Petrášek[1]**
*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Prague, Czech Republic*

E-mail of presenting author: skokan@ueb.cas.cz

Phytohormones regulate many developmental processes in land plants *(Embryophyta)*, both on cellular and tissue level. This regulation is expected to have been a milestone in plant evolution, as some of these processes must have been crucial for the earliest plants to make their transition from water to land. As for auxin, the key features of its metabolism, signalling and transport known from dicotyledons are all possessed by the most basal model land plant, a moss *Physcomitrella patens*. Attention has turned to freshwater green algae of clade *Streptophyta* (a sister group to land plants).
Here we focus on a member of genus *Spirogyra sp*. (CAUP strain K902) of the conjugating green algae *(Zygnematophyceae)*, which are being proposed by some as the closest living relatives to land plants. These algae form chains of cells with characteristic spiral chloroplasts. We have shown that only very high concentrations (compared to land plants) of a synthetic auxin 1-NAA (50 μM) promoted elongation of these cells. The natural auxin IAA did not exhibit this effect. Auxin transport assays were performed using a technique for suspension-cultured cells of vascular plants. Active influx of IAA, but not NAA was detected by using auxin influx inhibitor CHPAA and auxin competition assays. Interestingly, auxin efflux inhibitor NPA did not show any effect. The inhibitor of ABCB/PGP activities, gravacin, inhibited the influx of both IAA and NAA.
These results suggests that in *Spirogyra sp.* there exists a mechanism by which NAA in high concentrations could positively affect cell elongation. Moreover, auxin accumulation assays suggest the existence of active auxin transporters, possibly ABCB/PGP-related auxin influx carriers.
*This work was supported by the Czech Science Foundation, project GAP305/11/2476 (JP).*

## P2-11 To decipher the possible presence of auxin transport machinery in chloroplast

**Prashanth Tamizhselvan[1], Kifah Abushamsieh[2], Matias Zurbriggen[3], Vanesa Tognetti[1]**
*[1]Plant stress signaling and adaptation, CEITEC, Masaryk University, Brno, Czech Republic; [2]Mendel University, Brno, Czech Republic; [3]Centre for Biological signaling studies, University of Freiburg, Freiburg, Germany*

E-mail of presenting author: prashanthts1987@gmail.com
E-mail of corresponding author: tognetti@ceitec.muni.cz

Environmental stresses adversely affect plant growth and development leading to worldwide yield losses. The plant hormone and signaling molecule auxin is a key player in plant development and plays an important role in plant stress responses. Lesser photosynthesis is partially responsible for some of the observed symptoms in stress adapted plants. This symptoms such as growth retardation, reduced metabolism and increased antioxidant activities helps to maximize plant survival. Auxin modifies chloroplast structure, chlorophyll synthesis, nuclear photosynthetic genes expression, and chloroplast transcription in response to environmental changes[1]. Thus auxin could hypothetically affect the remodeling of the photosynthetic apparatus to minimize photo oxidative damage induced upon stress[1]. Reciprocally, chloroplasts synthesize tryptophan-derived precursors for IAA biosynthesis and in some plants can actively biosynthesize auxin[1]. Moreover, recently chloroplast redox state was shown to modulate auxin homeostasis [2]. These data show that there is a strong connection between chloroplast processes and auxin homeostasis. However, the connection between auxin and photosynthesis is rarely described.

To investigate whether the Arabidopsis chloroplast can transport auxin, chloroplast localized putative auxin transporters were selected. *In silico* promoter gene analysis shows that our target genes share some similarities with the promoters of ATP Binding Cassette auxin transporters. Auxin transport machinery in chloroplasts will be studied using a novel ratiometric auxin biosensor [3]. Owing to the vital importance of photosynthesis for plant growth and survival, understanding how chloroplast and auxin metabolism are interconnected will bring novel insights into plant stress biology applicable to improve the performance of crops under field conditions.

1. *Tognetti et al. (2011). Plant Cell & Environment. 35: 321-333.*
2. *Ferrández et al. (2012). Plant Signaling and Behavior. 7: 1177-1179.*
3. *Wend et al. (2013) Scientific Reports 3: 2052.*

## P2-12 A two-channel model for shoot auxin transport

**Martin van Rongen[1], Genevieve Hines[1], Tom Bennett[1], Ottoline Leyser[1]**
*[1]Sainsbury Laboratory, Cambridge University, Cambridge, UK*

E-mail of presenting author: mv372@cam.ac.uk

Polar auxin transport (PAT) is an essential component of shoot branching control. We have previously proposed a canalization-based mechanism to explain the observed role of PAT in bud activation and hence shoot branching. Phytomer-level computational modelling has demonstrated the plausibility of this idea, but cannot capture the full-range of behaviour observed in Arabidopsis. We therefore attempted to produce a refined cellular-level model of auxin transport in the shoot, based on improved understanding of auxin transport dynamics and expression of auxin transport proteins.

We used pulse assays to assess the progression of a pulse of radiolabelled auxin down *Arabidopsis* stem sections. Strikingly, these profiles show that the pulse's shape is not conserved as the pulse progresses down the stem. Instead it spreads out and flattens rapidly. Computer simulations of the pulse assays were unable to reproduce satisfactorily the experimental profiles if the stem was represented by a single, highly polarized auxin transport channel, as represented by PIN1-expressing cell files in the stem. Instead, the simulations captured auxin dynamics more successfully if the stem contained a main PAT channel that can exchange auxin with one or more channels with non-polar or less-polar auxin transport. To test this idea, we examined the localization of other PIN proteins in the stem and found that several PIN proteins localize in and around the PIN1-expressing cells.

Our results suggest that the tissue next to the main PAT channel could be an important reservoir of auxin, and that other auxin exporters than PIN1 must play a role in the lateral distribution of auxin across the stem. This role might be important in the initial canalization of the auxin exported from a bud towards the main stem PAT stream.

## P2-13 A forward genetic screen identified new regulators in auxin-dependent degradation of auxin transport proteins in Arabidopsis thaliana

**Radka Zemová[1], Hélène Robert[1], Marta Zwiewka[1], Jiří Friml[2]**
*[1]Mendel Centre for Genomics and Proteomics of Plant Systems, Kamenice 5, CEITEC-Masaryk University, Brno 625 00, Czech Republic;*
*[2]Developmental and Cell Biology of Plants, Institute of Science and Technology Austria (IST), 3400 Klosterneuburg, Austria*

E-mail of presenting author: radka.zemova@gmail.com
E-mail of corresponding author: Jiri.FRIML@ist.ac.at

The plant hormone auxin is a major player for the regulation of plant growth development including embryo and root patterning, lateral organ formation and growth responses to environmental stimuli. Auxin is polarly cell-to-cell transported by the action of specific auxin influx (AUXIN-RESISTANT1 (AUX1) proteins) and efflux (PIN-FORMED (PIN) proteins) carriers, whose subcellular localizations indicate the direction of the auxin flow. Auxin itself regulates its own transport by modulation of the expression and subcellular localization of the auxin transporters. Short auxin treatment activates the transcription of *PIN* and *AUX1* genes and stabilizes PIN proteins at the plasma membrane, whereas prolonged auxin application promotes the turnover of PIN proteins and their vacuolar degradation. In this study we took advantage of forward genetics, which opens up the possibility of identifying molecular components playing a role in these processes. In order to identify new mutants with impaired auxin transport or showing disorders with routing of auxin carriers, we used EMS mutagenized *Arabidopsis* transgenic line *PIN2::PIN2-GFP AUX1::AUX1-YFP eir1 aux1* and we looked for mutants with stronger fluorescent signals after prolonged treatment with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D). The detailed analysis of 3 candidate mutant lines will be presented.

*This work was supported by the Grant Agency of the Czech Republic (GA13-40637S.) and the European Regional Development Fund (CZ.1.05 /1.1.00/02.0068,CZ.1.07/2.3.00/20.0043)*

# SESSION 3: SIGNALING

## O3-1 Shoot branching and the auxin transport network

**Ottoline Leyser**[1]
[1]*Sainsbury Laboratory University of Cambridge, Bateman Street, Cambridge CB2 1LR, UK*

E-mail of presenting author: ol235@cam.ac.uk

According to the classical theory of Tsvi Sachs, the development of vascular strands can be induced along canals of active auxin transport that form by positive feedback between an initial auxin flux from an auxin source to an auxin sink, and the up-regulation and polarization of that flux. A good example of this process in action is the development of vascular connectivity between an activating axillary bud, an auxin source, and the main stem, an auxin sink. We have extended this idea to provide an explanation for the inhibition of bud activity by auxin moving in the main stem by proposing that canalization of auxin transport out of the bud is essential not only for vascular connectivity but also for sustained bud activity. This has led us to investigate in more detail temporospatial aspects of auxin transport between the bud and the stem. The expression of multiple auxin transporters in stem, and their divergent effects on auxin transport profiles suggest different transport properties for different stem tissues, with consequences for the dynamics of auxin transport in the stem and from the bud to the stem.

## O3-2 Novel targets of cytokinin signaling

**Thomas Schmülling**[1], **Silvia Nitschke**[1], **Anne Cortleven**[1], **Michael Riefler**[1], **Daniela Pezzetta**[1], **Stefanie Zintl**[1], **Jan-Erik Leuendorf**[1], **Wolfram Brenner**[1]
[1]*Dahlem Centre of Plant Sciences (DCPS), Institute of Biology/Applied Genetics, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: tschmue@zedat.fu-berlin.de

The availability of numerous cytokinin signaling mutants has enabled novel approaches to study cytokinin functions. The analysis of their behavior under diverse environmental conditions has revealed hitherto unknown roles of the hormone in responding to stressful conditions and in mediating diverse environmental cues. Examples will illustrate a function for cytokinin in the response to high light stress, as a negative regulator of the phyA-mediated very low fluence response in seed germination and in the avoidance of circadian stress. The data indicate a plethora of specific signaling cascades operating downstream of the two-component system linking cytokinin to different other pathways of the cellular signaling network to realize the hormone´s pleiotropic activities.

### O3-3 Auxin response specificity in plant development

**Dolf Weijers**[1]
[1]*Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands*

E-mail of presenting author: dolf.weijers@wur.nl

Hormone activity in multicellular organisms depends on the generation of specific local responses to general signals. A key question is what molecular mechanisms generate local response specificity and what cellular reprogramming underlies unique developmental output. Auxin controls a wide array of developmental processes, most of which are mediated by the DNA-binding AUXIN RESPONSE FACTORS (ARFs). All sequenced land plant genomes encode at least 10 ARF genes, and in Arabidopsis, these perform distinct functions by regulating different sets of genes. I will present recent progress from a combined proteomics, structural biology, genetics, transcriptomics and cell biology approach. I will discuss how intrinsic ARF properties and protein complexes determine target gene selection, and how these auxin responsive genes collectively determine auxin-controlled plant development.

### O3-4 Cytokinin Response Factors, branching out into stress and senescence

**Aaron Rashotte**[1]
[1]*Biological Science, Auburn University, Auburn, USA*

E-mail of presenting author: rashotte@auburn.edu

Cytokinin Response Factors or CRFs are a subgroup of AP2/ERF transcription factors that form a branch of the two-component cytokinin signaling pathway. While several CRFs were originally identified as cytokinin inducible genes, not all CRFs are transcriptionally regulated by cytokinin. However, most CRFs do show transcriptional regulation by various abiotic stresses, including salt, temperature, and oxidative stresses. Phylogenetic analysis of CRF proteins from across plants indicates that CRFs form a monophyletic group in Angiosperms that can be divided into five distinct clades (I-V), with every species having at least one CRF protein in each clade. Using this we have taken a functional genomic approach to reveal that each CRF clade appears to be uniquely regulated in a manner that is conserved among species. Analyses of cytokinin and various stress regulation have been examined in Arabidopsis and tomato CRFs to help further define these roles. One example of this is the clade III CRFs that are highly induced by cytokinin that have been linked to senescence response. Recently these genes in Arabidopsis were independently linked to oxidative stress, when they were shown to be direct targets of the mitochondrial retrograde response (MRR) signaling pathway members. Together this suggests that CRFs may serve as a convergent point between the cytokinin and MRR oxidative stress signaling pathways. Results related to showing this and the clade specific functional roles of CRF will be presented.

### O3-5 Characterization of a novel repressor of the transcriptional response to cytokinin

**Alexander Heyl[1], Andreas Pfeifer[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: alexander.heyl@fu-berlin.de

Cytokinins are plant hormones, which play a crucial role in many aspects of plant growth and development. At least for the model plant *Arabidopsis thaliana,* it was shown that most if not all of the cytokinin output is at least partially mediated by one class of transcription factors, the so-called type-B response regulators (RRs). However, other transcription factors were found to play a role in the transcriptional response to cytokinin indicating that this regulatory pathway is more complex than thought previously. In a genetic screen, we identified a small protein, which negatively regulates the cytokinin response. In protoplast transactivation assays, this protein strongly represses the transactivation activity of type-B RR, ARR1, and to a much lower level that of ARR2. In contrast, the function all other type-B RRs tested was not affected by the co-expression of the factor. Overexpression of the protein in the *arr10/arr12* mutant background partially phenocopies the *arr1/arr10/arr12* mutant, thus providing further evidence for the function of the protein also *in planta*. Experiments dissecting the biological role and the molecular mechanism of this novel repressor will be presented.

### O3-6 Mechanisms of ABP1-dependent cell expansion come to light slowly but surely

**Catherine Perrot-Rechenmann[1], Sébastien Paque[1], Laurie Grandont[1], David Alabadí[2], Miguel Blázquez[2], Grégory Mouille[3]**
*[1]Institut des Sciences du Végétal, Saclay Plant Science LabEx, CNRS, Gif sur Yvette, France; [2]Instituto de Biología Molecular y Celular de Planta, Universitat Politécnica de Valencia, Venecia, Spain; [3]Institut Jean-Pierre Bourgin, INRA, Versailles, France*

E-mail of presenting author: catherine.rechenmann@isv.cnrs-gif.fr

Cell expansion is an increase in cell size size and plays an essential role in plant growth and development. This complex process relies on multiple factors amongst which mechanical properties of the primary cell wall and phytohormones play major roles. The AUXIN BINDING PROTEIN 1 (ABP1) is known to be essential for the regulation of cell expansion however the mechanism by which this protein controls expansion remains unknown. We used dark grown hypocotyl as a model system for studying at various levels the contribution of ABP1 to cell expansion. Molecular and genetic evidences indicate that ABP1 affects the expression of a broad range of cell wall related genes mainly via the modulation of the $SCF^{TIR/AFBs}$ signaling pathway. Multiscale analysis of the cell wall after functional inactivation of ABP1 revealed that the protein is required for remodelling of hemicellulose xyloglucan structure which is required for cell wall expansion. In addition, ABP1 is involved in the organization of cortical microtubules and cellulose microfibrils that are also susceptible to affect cell expansion.

### O3-7 The Bioenergetics of auxin binding to TIR1

**Richard Napier[1], Mussa Quareshy[1], Veselina Uzunova[1], Gareth Price[1], Noel Ferro[3], Stefan Kepinski[2]**
*[1]School of Life Sciences, University of Warwick, Coventry, UK; [2]Plant Science, University of Leeds, Leeds, UK; [3]Institute for Theoretical Chemistry, Bonn, Germany.*

E-mail of presenting author: richard.napier@warwick.ac.uk

Auxin binding to the receptor TIR1 permits binding of the Aux/IAA co-receptor proteins, thereby promoting their ubiquitination and consequent degradation. The structure of the co-receptor complex has been solved at 2.5Å and used both as the template for models of AFB5 (Tan et al., 2007; Calderon et al., 2012) and as an aid in the design of small molecule agonists and antagonists (Hayashi et al., 2008). Structure-activity relationships for co-receptor assembly have been measured by surface plasmon resonance and combined with chemometrics to begin to dissect the molecular basis of auxin specificity (Lee et al. 2013). In an extension of this work, drug docking algorithms have been applied to the TIR1 and AFB5 binding sites. The results suggest that selectivity is not readily defined by the coordinates of the binding pocket alone. TIR1 flexibility may play a significant role in both auxin and Aux/IAA interactions, even though little conformational change was seen in the crystal structures. We are using thermodynamic measurements combined with various simulations of domain mobility, molecular dynamic and quantum calculations to test our ideas and to help define the very earliest events of auxin perception in terms of their energetics and mechanism.

### O3-8 Evidences for the initiation of cytokinin signaling in the endoplasmic reticulum

**Sergey Lomin[1], Yulia Myakushina[1], Dmitry Arkhipov[1], Vladimir Popenko[2], Olga Leonova[2], Thomas Schmülling[3], Georgy Romanov[1]**
*[1]Signaling Systems Laboratory, Institute of Plant Physiology RAS, Moscow, Russian Federation; [2]Cell Biology Laboratory, Institute of Molecular Biology RAS, Moscow, Russian Federation; [3]Institute of Biology/Applied Genetics, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: losn1@yandex.ru

Cytokinin receptors were shown recently to be located mainly in the endoplasmic reticulum (ER). However, it remained unclear whether the ER-located receptors are active and the presence of a minor part of receptors in the plasma membrane (PM) could not be excluded. As ER and PM differ in composition and properties, the activity of receptors might depend on their local surrounding. We have, therefore, checked the functionality of receptors located in different membranes. Firstly, we tested receptor topology by a protease protection assay. The cytoplasmic part of the AHK3 receptor was shown to be located in the cytosol and the hormone binding domain in the ER lumen. This topology is consistent with signal transduction from ER membranes. To check the subcellular localization of receptor-phosphotransmitter interaction in planta we performed BiFC experiments. Receptor and phosphotransmitter genes were fused with split eYFP sequences, expressed in *Nicotiana benthamiana* leaves and the subcellular localization of protein interaction detected by confocal microscopy. We found that receptors interact with phosphotransmitters at the ER network and around nuclei, an interaction pattern being similar to receptor localization. Finally we tested the functionality of receptors in different membranes by an in vitro kinase assay visualizing the phosphorylation of phosphotransmitter proteins. Receptor genes were expressed in *N. benthamiana* leaves and ER and PM fractions were obtained from leaf homogenate by ultracentrifugation in a step sucrose gradient. Phosphotransmitters were obtained by expression of corresponding genes in *E. coli* followed by affinity column purification. Kinase assays were performed in a mixture of membranes, phosphotransmitters, and $\gamma$-$^{32}$P-ATP as substrate in the presence of various *trans*-zeatin concentrations followed by SDS-PAGE and blotting onto PVDF membranes. We found that the bulk of cytokinin-dependent kinase activity belonged to ER fractions indicating that ER-located receptors are active.

### O3-9 Elucidating auxin signaling by next generation phenotyping of rapid auxin responses

**Hartwig Luethen[1], Simon Fraas[1], Daniel Schenck[1]**
*[1]Physiology, Biozentrum Flottbek of the University, Hamburg, Germany*

E-mail of presenting author: h.luthen@botanik.uni-hamburg.de

We studied the impact of receptor and signaling mutations on the time courses of rapid auxin growth responses. The astonishing finding was that the *tir1 – afb* mutations had only a very faint influence on hypocotyl elongation, while the rapid root growth inhibition was strongly affected. Knocking out or overexpressing *twisted-dwarft 1*, an interaction partner of the ABCB auxin transporters, had a massive effect on auxin sensitivity. Based on these findings, we will discuss the roles of TIR1/AFB and the ABCBs in auxin signaling.
We feel that much of the confusion in the understanding of the rapid growth responses in roots and hypocotyls could be overcome by establishing an easy-to-operate high throughput system for analyzing the rapid growth responses at high time resolution. We will present a scanner-based imaging system for recording the responses in a large number of experiments simultaneously. We will also demonstrate our software Hansa Trace, which we developed to analyse the images rapidly and with a minimum of user interaction. The software detects and measures root and hypocotyl growth responses using a novel 2D skeletonizing algorithm. A large number of relevant mutants can now be fully characterized in a kind of physiological response phenotyping.

### O3-10 Immunological manipulation of intracellular cytokinin distribution determines endoplasmic reticulum as a cellular compartment responsible for cytokinin signal perception.

**Zuzana Gelová[1], Petra ten Hoopen[2], Ondřej Novák[3], Václav Motyka[4], Udo Conrad[2], Lubomír Janda[5], Jan Hejátko[1]**
*[1]Functional Genomics and Proteomics of Plants, Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [2]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; [3]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Palacký University, Olomouc, Czech Republic; [4]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Praha, Czech Republic; [5]NMR Spectroscopy, Central European Institute of Technology, Masaryk University, Brno, Czech Republic*

E-mail of presenting author: zuzana.gelova@email.cz
E-mail of corresponding author: hejatko@sci.muni.cz

Binding of cytokinins (CKs) to the extracellular CHASE domain of sensor histidine kinases (HKs) AHK2, AHK3 and AHK4 acting as CK receptors is supposed to activate the downstream multistep phoshorelay pathway transducing the CK signal into nucleus. Recent studies suggested localization of CK receptors to endoplasmic reticulum (ER). However, the functional importance of ER-localized CK receptors remained elusive.
Here we employed immunomodulation, a molecular tool based on ectopic expression of genes encoding recombinant single-chain variable fragments (scFvs) in manipulating cellular distribution of CK trans-zeatin riboside (tZR). We screened Tomlinson Human I+J scFv Library by tZR conjugated with BSA, selected specific scFv recognizing tZR (αtZR scFvs) and prepared *Nicotiana tabaccum* stable lines with ectopically expressed αtZR scFvs targeted to endoplasmatic reticulum (ER) (35S:KDEL-αtZR_scFv). In 35S:KDEL-αtZR_scFv lines we observed increased sensitivity to CK-mediated reduction of the root apical meristem size and increased number of dividing cells in the shoot apical meristem, suggesting CK over-sensitizing response. Accordingly, in 35S:KDEL-αtZR_scFv lines we observed upregulation of CYTOKININ OXIDASE/DEHYDROGENASE activity, one of the first responses aiming to recover the CK homeostasis. Immunolocalization using KDEL specific antibodies proven the ER localization of αtZR scFvs in 35S:KDEL-αtZR_scFv transgenic lines. Using transient expression of TCS:LUC reporter in tobacco protoplasts we showed the ability of 35S:KDEL-αtZR_scFv construct to upregulate CK signaling. Altogether, our results strongly suggest that increasing the pool of ER-located CKs upregulates CK signaling leading to CK over-sensitizing response and providing thus the first evidence for the functional importance of ER-located CKs in plants.
*Supported by the Czech Science Foundation (P305/11/0756) and European Regional Development Fund (Central European Institute of Technology project no. CZ.1.05/1.1.00/02.0068).*

## O3-11 Characterization of the five cytokinin receptors in apple tree and their implications in biotic interactions

**Gaëlle Glevarec[1], Dimitri Daudu[1], Elsa Allion[1], Nicolas Papon[1], Vincent Courdavault[1], Audrey Oudin[1], Arnaud Lanoue[1], Thomas Dugé de Bernonville[1], Emilien Fourreau[1], Céline Melin[1], Sabine Carpin[2], Marc Clastre[1], Martine Courtois[1], Marie-Noëlle Brisset[3,4,5], Emilie Vergne[3,4,5], Bruno Le Cam[3,4,5], David Giron[6], Grégory Mouille[7,8], Stéphanie Boutet-Mercey[7,8], Nathalie Giglioli-Guivarc'h[1], Joël Crèche[1], Sébastien Besseau[1]**
*[1]EA 2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 31 av. Monge, F37200 Tours, France; [2]Université d'Orléans, UFR-Faculté des Sciences, UPRES EA 1207, Laboratoire de Biologie des Ligneux et des Grandes Cultures (LBLGC), BP 6759, F-45067 Orléans, France; [3]INRA, UMR1345 Institut de Recherche en Horticulture et Semences, F-49071 Beaucouzé, France; [4]Université d'Angers, UMR1345 Institut de Recherche en Horticulture et Semences, SFR 4207 QUASAV, PRES L'UNAM, F-49045 Angers, France; [5]AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, F-49045 Angers, France; [6]Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS – Université François-Rabelais de Tours, UFR Sciences et Technique, 37200 Tours, France; [7]INRA UMR1318 Inst. J.-P. Bourgin, Saclay Plant Sicences, 78026, Versailles Cedex, France; [8]AgroParisTech UMR1318 Inst. J.-P. Bourgin, Saclay Plant Sciences, 78026, Versailles Cedex, France*

E-mail of presenting author: gaelle.glevarec@univ-tours.fr

Cytokinins (CK) play major roles in many different developmental and physiological processes and recently their implication in plant-pathogen interactions has been established. The characterization of CK signaling is a prerequisite to understand CK role in response to biotic stress. In plants, CK signaling pathway involves a phosphotransfer cascade composed by histidine-kinase receptors (CHK), histidine phosphotransfer protein (HPt), response regulators (RR) and CK-response factors (CRF). We initiated a study of CK signaling in apple tree (*Malus x domestica)* challenged with pathogens (*Erwinia amylovora* and *Venturia inaequalis*, the causal agents of fire blight and apple scab respectively) or pest (*Phyllonorycter blancardella,* the leaf-miner insect). We first isolated five CK receptors named MdCHK2, MdCHK3a, MdCHK3b, MdCHK4a and MdCHK4b and analysed their transcript abundance in plant organs. Then, we studied their expression patterns in response to CK and the biotic stresses cited above. Finally, we evaluated the substrate specificity and sensitivity in bioassays in which conditional survival of Saccharomyces *sln1* mutant strains expressing various MdCHKs depends on the presence and perception of CK. Our results highlight that each receptor presents a specific gene expression profile in apple tree organs and in response to pathogens. Each MdCHK also possesses specific sensitivity to different types of CK (including CK that could be secreted by pathogens). Taken together, our data suggest that MdCHKs could display distinct functions for the CK perception in apple tree and that physiological processes in their response to biotic stresses could be primarily propagated by MdCHK receptors.

## O3-12 Summarizing a decade of cytokinin-regulated transcriptome analysis

**Wolfram G. Brenner[1], Thomas Schmülling[1]**
*[1]Applied Genetics, Dahlem Centre of Plant Science (DCPS), Institute of Biology, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: wbrenne@zedat.fu-berlin.de
E-mail of corresponding author: tschmue@zedat.fu-berlin.de

The transcriptional response of the model organism *Arabidopsis thaliana* to cytokinin was investigated by different research groups as soon as large-scale transcriptomics became affordable. The flood of data started in 2002 with a publication addressing shoot induction in calli by cytokinin (Che et al., 2002), followed by two studies dedicated to gene expression after cytokinin treatment of seedlings (Rashotte et al., 2003; Brenner et al., 2005), the latter using the full-genome ATH1 GeneChip®. Since then, many more large-scale transcriptomic datasets relating to cytokinin have been generated using different technological platforms, some of which are published only in databases (Brenner et al., 2012), culminating in a study employing RNA sequencing (Bhargava et al., 2013). Based on this body of data, several approaches have been made to establish a core set of cytokinin-regulated transcripts by meta-analysis of transcriptomic data using different preferences regarding the datasets used (Brenner et al., 2012; Bhargava et al., 2013). We have now added another meta-analysis derived from an independent microarray technology (CATMA) and compared all the meta-analyses available with the RNA-sequencing data to establish the best possible core set of cytokinin-regulated transcripts. Remarkably, the biological and molecular functions of more than 50 % of the 66 identified core genes is still unknown, while most of the other genes play a role in developmental processes, response to stress and stimuli, and signal transduction. Based on recently published data on binding sites of type-B response regulators (Franco-Zorrilla et al., 2014), we identified those that are significantly enriched in cytokinin-responsive promoters and are probably important in recruiting the transcription factors to the promoters. These novel sequence motifs, if confirmed as functional *cis* elements, may improve the definition of a cytokinin-responsive promoter.

### O3-13 Heterogeneous intra- and extra-cellular distribution of cytokinins in Arabidopsis roots

**Ioanna Antoniadi[1], Ondrej Novak[2], Bruno Muller[3], Karin Ljung[1], Colin Turnbull[4]**
*[1]Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Umeå, Sweden; [2]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany AS CR & Palacký University, Olomouc, Czech Republic; [3]Institute of Plant Biology, Zurich-Basel Plant Science Center, University of Zurich,, Zurich, Switzerland; [4]Life Sciences, Imperial College London, London, UK*

E-mail of presenting author: ioanna.antoniadi@slu.se

TCSn::GFP is a new synthetic promoter fusion which enables reporting of cytokinin transcriptional responses at the cellular level. It shows a heterogeneous distribution of expression in the primary root apex of Arabidopsis seedlings, suggesting a modulation of cytokinin content at the cellular level.
In order to understand the biological relevance of the TCSn::GFP reporter line, we performed a cell-type specific validation of the reporter. Analytical approaches, such as liquid chromatography – tandem mass spectrometry (LC-MS/MS), can provide accurate quantitative measurements of cytokinin metabolites. Here, we used a novel method combining Fluorescence Activated Cell Sorting (FACS) with ultra-sensitive LC-MS/MS analysis to quantify cytokinin metabolites in the GFP+ and GFP- cells of TCSn::GFP root tips. The most abundant metabolites detected in the GFP+ cells were the cytokinin glucosides, not the bioactive cytokinins. Since FACS is a method that requires protoplast isolation and therefore excludes cell walls and apoplastic space, we also measured cytokinin levels in the apoplastic fluid of the roots. To our surprise, a large proportion of the cytokinin nucleobases and ribosides were present in the apoplastic fraction, while the cytokinin glucosides were predominantly found in the symplastic fraction in accordance with our sorting data. Even though cytokinin receptors are believed to be located mainly at the endoplasmic reticulum, our findings suggest that a large portion of the active cytokinin metabolites are found in the apoplast. This implies significant roles for cytokinin transporters and/or cytokinin receptors resident on the plasma membrane.
We believe that these findings will be relevant to many recent studies where the TCS reporter has facilitated the discovery of new cytokinin functions and enhanced our understanding of existing ones.

### O3-14 Auxin-mediated secretion of the ABP1 receptor to the cell surface

**Xu Chen[1], Peter Grones[1], Riet De Rycke[2], Jisheng Chen[3], Krzysztof Wabnik[1], Deshu Lin[3], Hana Rakusova[1], Catherine Perrot-Rechenmann[4], Zhenbiao Yang[3], Jiri Friml[1]**
*[1]Plant Cell Biology, Institute of Science and Technology Austria, Klosterneuburg, Austria; [2]Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium; [3]Department of Botany and Plant Sciences, University of California Riverside, California, USA; [4]UPR2355 CNRS, Institut des Sciences du Végétal, Cedex, France*

E-mail of presenting author: xu.chen@ist.ac.at

Auxin, an essential plant hormone and coordinates many aspects of plant growth and development. AUXIN BINDING PROTEIN1 (ABP1) is one of the first characterized extracellular receptors and has been shown to mediate rapid responses to auxin. ABP1 predominately resides in the endoplasmic reticulum (ER) but the minor, physiologically relevant fraction of ABP1 escapes from the ER to the extracellular space by a yet unknown mechanism. Here, we identified an ER-localized and cell surface-docking glycosylphosphatidylinositol (GPI)-anchored SKEWED5 (SKU5) protein that binds ABP1 in an auxin-dependent manner.*sku5* mutants, in particular inactivation of *SKU5* homologues (SKSs) phenocopy abp1 mutant lines. The secretion of SKU5 to the cell surface requires ABP1-dependent auxin signaling and vice versa SKU5 assists ABP1 secretion to the cell surface. These findings identified a novel mechanism, SKU5 and its homologues regulate in auxin-dependent manner the capacity ofextracellular auxin perception via its cell surface receptor ABP1.

### O3-15 The importance of auxin binding for ABP1 function

**Peter Grones[1], Jiri Friml[1]**
*[1]Developmental and Cell Biology of Plants, IST Austria, Klosterneuburg, Austria*

E-mail of presenting author: peter.grones@ist.ac.at

One of the key growth regulators in plants is the plant hormone auxin. Auxin mediates a number of developmental and growth responses in plants including cell elongation, cell division, cell differentiation, required for organ initiation and patterning, tropic growth responses, leaf senescence, cell and tissue polarization (Davies et al., 1987). Auxin Binding Protein1, ABP1, was one the first proteins identified to have a high specificity and affinity for auxin binding. While ABP1 has been identified several decades ago, surprisingly little is known about its mode of action. The majority of the protein is localized in the ER, with a fraction escaping to the apoplast where it is supposed to act at the outer side of the plasma membrane. Few years ago, abp1-5 allele, possessing a mutation in a conserved auxin binding pocket, which resulted in partial auxin resistance, was identified (Xu et al., 2010).
Based on the crystal structure of ABP1 protein (Woo et al., 2002) we designed a mutant version of this protein in which several essential residues required for auxin binding, were substituted. The resulting transgenic lines were subjected to detailed morphological and cell biological analyses that helped us to unravel importance of auxin binding to ABP1 for variety of its biological functions.

### O3-16 AUXIN-BINDING-PROTEIN 1 (ABP1) Controls Auxin- and Red Light-Induced Gene Expression and Physiologies in Arabidopsis

**Günther Scherer[1], Yunus Effendi[1], Noel Ferro[2], Markus Geisler[3]**
*[1]Naturwissenschaftliche Fakultät, Molekulare Ertragsphysiologie, Leibniz Universität Hannover, Hannover, Germany; [2]Mulliken Center for Theoretical Chemistry, Institute for Physical and Theoretical Chemistry, University of Bonn, Bonn, Germany; [3]Department of Biology – Plant Biology, University of Fribourg, Fribourg, Switzerland*

E-mail of presenting author: scherer@zier.uni-hannover.de

The function of the extracytoplasmic AUXIN-BINDING-PROTEIN1 (ABP1) is largely enigmatic. We complemented a homozygous T-DNA insertion null mutant of *ABP1* and generated thus four genetically engineered *abp1* mutants. Based on *in silico* modeling, the *abp1* mutants were suggested to have different geometries of the auxin binding pocket and calculated binding energies lower than wild type (wt). Mostly, functions linked to auxin transport(gravitropism, phototropism, polar transport in the root, apical dominance) and auxin sensitivity of lateral root induction were compromised inthese *abp1* mutants. Auxin did not induce hypocotyl elongation in the light in wt and *abp1* mutants but in the dark elongation was found and was ABP1-dependent. Unexpectedly, red light functions, such as elongation of hypocotyls in constant red (R) and far red (FR) light, in physiological shade, and inhibition of gravitropism by R or FR, were also compromised in these *abp1* mutants. Using auxin- or light-induced expression of marker genes we show that auxin-induced expression is delayed in *abp1* mutants already after 10 min. ABP1 at the plasma membrane cannot be modulated in 10 min by transcription-based mechanisms so that ABP1is placed functionally upstream of TIR1. Light-induced expression in *abp1* mutants is modulated within 60 min even though TIR1/AFB or phyB are thought to act as receptors relevant for gene expression regulation. Testing expression of selected marker genes responding to both auxin and shade in seedlings shows that for both stimuli regulation of marker gene expression was different after already 10-20 min in wt and *phyB*. These rapid expression responses show that ABP1 and phyB trigger interwoven signaling pathways.

### O3-17 Looking for a needle in the haystack – scrutinizing the ABP1 protein structure for ways by which it might signal the binding of auxin

**Lindy Abas**[1]
[1]*Applied Genetics and Cell Biology, Universität für Bodenkultur Wien, Vienna, Austria*

E-mail of presenting author: melinda.abas@boku.ac.at

Auxin Binding Protein 1 (ABP1) has been a long-running candidate for the position of auxin receptor in plants ever since its discovery in the 1970s and 80s. The campaign continues until today and ABP1 has many enthusiastic supporters, but the precious title somehow always has to be defended due to many peculiarities that have not yet been satisfactorily resolved. The beautiful protein crystal structure of maizeABP1 was published over 10 years ago thanks to the efforts of the Napier and Pickersgill groups, who managed to solve it both in the presence and absence of bound auxin (Woo et al 2002). Surprisingly, no conformational changes were seen between the two states, so although the structures showed clearly how auxin (NAA) was positioned in the binding pocket, they were unfortunately not informative in revealing a precise mechanism by which the protein may signal the binding of auxin. However, it was suggested by the authors that crystal contacts may have prevented the mobility of the C-terminus, a domain that has previously also been suggested as a possible candidate for signal transduction (Leblanc et al 1999). Short (5ns) molecular dynamics simulations of the ABP1 structure have since reported possible movements in the C-terminus, the extent of which was affected by the presence of an auxin ligand (Bertoša et al 2008). Here, I will present an analysis of the ABP1 crystal structure to consider the C-terminus and also to explore other theoretical alternatives as to how auxin binding could affect the conformation of ABP1.

## POSTERS

### P3-1 A Dual specificity Protein Phosphatase (DsPTP1) Positively Regulates Auxin Signaling through the Inhibition of a MAP kinase in Arabidopsis

**Woo Sik Chung**[1], **Kyung Eun Kim**[1], **Jonguk An**[1], **Hay Ju Han**[1], **Sun Ho Kim**[1]
[1]*Division of Applied Life Science (BK21+ Program) and Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, South Korea*

E-mail of presenting author: chungws@gnu.ac.kr

Auxin regulates many aspects of growth and development in plants. Several studies have reported that components of MAPK signaling pathways are involved in auxin signaling. In this study we showed that MAP kinase is involved in the stabilization of a repressor of auxin signaling. One of MAP kinases, MPK12, is known to be regulated by a protein phosphatase, IBR5. We identified a calmodulin binding dual-specificity protein phosphatase, DsPTP1, as another upstream regulator of MPK12. We found that DsPTP1 could directly bind to MPK12 in protein pull-down assay, luciferase complementation imaging assay and co-immunoprecipitation assay. As expectedly, DsPTP1 was able to inactivate the active MPK12 by dephosphorylation. To identify the biological function of DsPTP1 in auxin signaling, we established DsPTP1 overexpressing transgenic plants. DsPTP1 overexpressing transgenic plants are more sensitive to exogenous auxin than wild type in primary root growth. To examine whether calcium signaling controls auxin signaling, we generated transgenic plants overexpressing a CaM binding negative mutant form of DsPTP1. Consequently, a CaM binding negative mutant form of DsPTP1 OX is more sensitive to exogenous auxin than DsPTP1 OX in primary root growth. These results suggest that not only IBR5 but also DsPTP1 is involved in auxin signaling through the regulation of MPK12. In addition, we suggest that calcium signaling may be involved in auxin signaling through the regulation of a calmodulin regulated protein phosphatase.

### P3-2 Exploring Mechanisms of Molecular Recognition between Histidine-Containing Phoshotransfer Protein AHP2 and Sensor Histidine Kinase CKI1 in the Multistep Phosphorelay Signaling from Arabidopsis thaliana

**Radka Dopitová[1], Oksana Degtjarik[1], David Řeha[2], Sandra Puehringer[3], Olga Otrusinová[1], Manfred S. Weiss[3], Lukáš Žídek[1], Ivana Kutá-Smatanová[2], Lubomír Janda[1], Jan Hejátko[1]**
*[1]Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic; [2]Inst. of Nanobiology and Structural Biology GCRC, Academy of Sciences of the Czech Republic, Nové Hrady, Czech Republic; [3]Helmholtz-Zentrum Berlin for Materials und Energy BESSY-II, Berlin, Germany*

E-mail of presenting author: radka.dopitova@gmail.com

In *Arabidopsis*, His-phosphotransfer proteins (HPts) play a role of signal transmitters from diverse sensor histidine kinases to response regulators within multistep phosphorelay pathway (MSP). The previously reported ability of HPts to interact with receiver domains of individual sensor histidine kinases with different affinities indicates certain specificity of the interaction between the two partners. In order to explore determinants of the interaction specificity between AHP2 and the receiver domain of CKI1 ($CKI1_{RD}$) at atomic resolution, we solved the three-dimensional structure of AHP2 by experimental phasing at 2.53 Å. Molecular dynamic simulations for 100 ns were applied to identify the key residues responsible for the AHP2-$CKI1_{RD}$ interaction. The AHP2-$CKI1_{RD}$ interaction was confirmed by NMR measurements and resulting chemical shift changes partially overlapped with the model. AHP2-$CKI1_{RD}$ model reveals strong protein-protein complex; the comparison of the model with recently published crystal structure of AHP1-$AHK5_{RD}$ suggests distinct differences in binding interface between both complexes, mostly in the amino acid residues mediating hydrophilic interactions. Due to the fact that the vast majority of interacting residues in AHP1 and AHP2 are represented by highly conserved residues, small structural differences of both AHPs and $AHK_{RD}$s are likely responsible for specific molecular recognition between both partners, as could be seen by our structural and bioinformatical comparisons.

*Supported by CZ.1.05/1.1.00/02.0068, ME ČR ME09016, GAČR 521/09/1699 and P305/11/0756, CZ.1.05/2.1.00/01.0024 and by the AS ČR AVOZ60870520.*

### P3-3 Engineering of the ligand specificity of the cytokinin receptor CRE1/AHK4 using site-directed mutagenesis

**Lucia Gallová[1], Karel Berka[2], Pavel Mazura[3], Václav Bazgier[1], Alexander Heyl[4], Lukáš Spíchal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University Olomouc, Šlechtitelů 11, Olomouc, Czech Republic; [2]Department of Physical Chemistry, Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacký University Olomouc, 17. Listopadu 12, Olomouc, Czech Republic; [3]Laboratory of Plant Molecular Biology, Institute of Biophysics AS CR and CEITEC– Central European Institute of Technology, Mendel University in Brno, Zemědělská 1, Brno, Czech Republic; [4]Department of Applied Genetics, Freie Universität Berlin, Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Albrecht-Thaer-Weg 6, Berlin, Germany*

E-mail of presenting author: lucia.gallova01@upol.cz

Cytokinins activate histidine kinase receptors to initiate their signalling pathway through binding to a CHASE domain of the receptor. Amino acid sequence of the CHASE domains of cytokinin receptors are highly conserved among plant species. The molecular basis of the ligand binding and recognition has been described only recently using co-crystallisation of CRE1/AHK4 sensor domain with cytokinins. Based on this knowledge we selected amino acids that can be responsible for the main differences in the ligand specificity of AHK3 and CRE1/AHK4, the receptors with the most contrasting ligand specificities. Using side-directed mutagenesis we introduced point mutations within the CHASE domain of CRE1/AHK4 to influence its affinity towards sensing of the cytokinin ligands preferred by AHK3. Receptor mutants were subsequently tested for their affinity in a competition assay using *E. coli* cells.

### P3-4 Interconnection between osmosensing and cytokinin signaling pathways in populus

**François Hericourt[1], Léopold Baquet[1], Mélanie Larcher[1], Françoise Chefdor[1], Inès Djeghdir[1], Christiane Depierreux[1], Franck Brignolas[1], Sabine Carpin[1]**
*[1]LBLGC, Université d'Orléans, Orléans, France*

E-mail of presenting author: francois.hericourt@univ-orleans.fr

The osmosensing pathway in *Arabidopsis thaliana* is constituted by a multi-step phosphorelay similar to the one of *Saccharomyces cerevisiae*, involving a Histidine-aspartate Kinase (HK) osmosensor, AHK1, and a Histidine-containing Phosphotransfer (HPt) protein, AHP2. In *Populus*, we have identified a cDNA encoding a HK, named HK1, and ten cDNAs encoding HPt proteins, HPt1 to HPt10. Interactions analysis between HK1 and all HPts in a two-hybrid system and *in planta* co-expression analysis revealed that three HPts are preferential partners of the osmosensor HK1, namely HPt2, HPt7 and HPt9.
In *A. thaliana,* AHP2 interacts not exclusively with AHK1 but also with AHK2, AHK3 and AHK4, which are involved in the cytokinin signaling pathway. In order to determine the interconnectivity between these two different signaling pathways in *Populus*, we conducted an exhaustive interaction study between HPts and cytokinin receptors.
Therefore, the homologous receptors of AHK2, AHK3 and AHK4 have been isolated from *Populus* and the cytoplasmic domain of these proteins has been tested in a two-hybrid system for their potential interaction with HPt1 to HPt10. The results suggest that some HPts could be commonly used by the two signaling pathways such as HPt2, HPt6 and HPt9. On the other hand, some others could have preferential signaling pathway involvement such as HPt1 and HPt3 in cytokinin pathway and HPt7 in osmosensing pathway.

### P3-5 New insights into the evolution of cytokinin receptors

**Alexander Heyl[1], Mhyheedeen Halawa[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: alexander.heyl@fu-berlin.de

Cytokinins are a class of plant hormones involved in many developmental and physiological processes. The cytokinin signal transduction is based on the two-component system, a signaling system which is also widespread among prokaryotes. An in depth phylogenetic analysis using a wide variety of different species ranging from bacteria and algae to modern land plants identified numerous putative cytokinin receptors. In addition to the clade of well known, "classical" cytokinin receptors, this bioinformatics analysis revealed a new class of putative cytokinin receptors, which contains only members from *Marchantia polymorpha* and *Physcomitrella patens*. Furthermore a third clade was detected with receptors from cyanobacteria, amoebe and chlorophyceae algae. To analyze the evolution of the cytokinin perception, the cytokinin binding activity of two receptors from the new subfamily, one from a moss (*Physcomitrella patens*) and one from liverwort (*Marchantia polymorpha*) and three receptors from the third clade, namely from cyanobacteria (*Synechocystis sp. PCC 6803)*, from amoebe (*Dictyostelium discoideum*) and from the chlorophyceae algae (*Chlamydomonas reinhardtii*) were characterized using different assays, such as *in vivo* hormone binding assays and *E. coli* complementation assays. The results of these experiments will be presented.

### P3-6 Signal integration and specificity in the multistep phosphorelay via protein-protein interactions

**Hrdinová Vendula[1], Horák Jakub[1], Klumpler Tomáš[2], Janda Lubomír[1], Hejátko Jan[1]**
*[1]Functional Genomics and Proteomics of Plants, Ceitec-MU, Brno, Czech Republic; [2]X-ray Diffraction and Bio-SAXS Core Facility, Ceitec-MU, Brno, Czech Republic*

E-mail of presenting author: v.hrdinova@email.cz

Multistep phosphorelay (MSP) represents important sensing mechanism in higher plants. MSP mediates wide range of regulatory processes triggered by phytohormones like cytokinins (CKs), or environmental stimuli like osmoregulation. In *Arabidopsis thaliana* there exist 9 histidine kinases (AHKs) containing both histidine kinase and receiver domain, which may be capable of transducing signal on 6 histidine-containing phosphotransfer proteins (AHPs). Thus, the specific interactions between AHPs and AHKs could represent a potent mechanism determining the signal integration on one side and specificity of cellular responses to different signals on the other one within the complex MSP network.
Here we tested interactions of receiver domain (RD) fragments of AHKs with AHPs by yeast-two hybrid assay (Y2H) using different interaction selective media to distinguish even the subtle differences between the strength of interaction. To confirm the observed specificity in planta we employed bimolecular fluorescence complementation (BiFC). Besides identification of new interactions, we demonstrate that each $AHK_{RD}$ recognizes specific subset of AHPs characterized with different strength of interaction. Importantly we found out that CK receptor-specific receiver-like domain of AHK4 ($AHK4_{RLD}$) or even its C-terminal alpha helix abolishes the ability of $AHK4_{RD}$ to interact with AHPs. In contrast, the absence of RLD increases the ability of $AHK4_{RD}$ to recognize AHPs. Using tertiary structure model-based prediction we suppose that $AHK4_{RLD}$ interacts with $AHK4_{RD}$ and we confirmed this interaction by Y2H. We propose that molecular recognition between AHPs and $AHK_{RD}$s determines the signaling specificity and integration in MSP and that $AHK4_{RLD}$ has a regulatory role in the process of molecular recognition of its downstream partners.
*Supported by CEITEC CZ.1.05/1.1.00/02.0068 and GACR P501/11/1150.*

### P3-7 LBD16 and LBD18 Are Linked to Auxin Influx Carriers LAX3 and AUX1 to Control Lateral Root Development in Arabidopsis

**Jungmook Kim[1], Han Woo Lee[1]**
*[1]Bioenergy Science and Technology, Chonnam National University, Gwangju, South Korea*

E-mail of presenting author: jungmkim@jnu.ac.kr

Auxin regulates lateral root (LR) development through a variety of transcriptional regulators including several members of *LBD/ASL* gene family in *Arabidopsis*. Here we demonstrate that *LBD16/ASL18* and *LBD18/ASL20* are linked to auxin influx carriers AUX1 and LAX(Like-AUX1)3 to control LR development in *Arabidopsis*. *lbd16* or *lbd18* mutation did not significantly alter LR emergence decreased by *lax3* mutation, whereas *LBD18* overexpression rescued the defect in LR emergence in *lax3* to the wild-type levels with concomitant overexpression of the *LBD18* target genes, *EXPANSIN14* (*EXP14*) and *EXP17*, and *POLYGALACTURONASE* regulated by *LAX3* with exogenous auxin. Genetic analyses indicated that *AUX1* requires *LBD16* and *LBD18* function for LR initiation and early stages of LR primordium (LRP) development. Analyses of *lbd16 lbd18 lax3 aux1* quadruple mutants implicated *LBD16* and *LBD18* function in LRP development. Expression of LBD18-SRDX in *lbd18* mutant inhibited LR initiation events, periclinal divisions, and LRP development in response to a gravitropic stimulus. LBD18-SRDX in *lbd18* suppressed promoter activities of the cell cycle genes *CDKA1;1* and *CYCB1;1*, whereas LBD18 activated expression of *CDKA1;1* and *CYCB1;1*. Taken together, these results suggest that *LBD18* controls LR emergence downstream of *LAX3* and that *LBD16* and *LBD18* regulate LR initiation and LRP development with *AUX1*.

### P3-8 Cytokinin receptor properties assessed in a plant membrane test system

**Sergey Lomin[1], Mikhail Steklov[1], Thomas Schmülling[2], Georgy Romanov[1], Dmitry Krivosheev[1]**
*[1]Signaling Systems Laboratory, Institute of Plant Physiology RAS, Moscow, Russian Federation; [2]Institute of Biology/Applied Genetics, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: gromanov@yahoo.com
E-mail of corresponding author: losn1@yandex.ru

Cytokinin receptors play a key role in cytokinin-dependent processes underlying plant growth, development and stress responses, therefore studies of functional properties of the receptors are of great importance. Previously these receptor properties were investigated in heterologous test systems using transformed unicellular microorganisms, mainly bacteria, expressing receptor proteins. However, within microorganisms, receptors reside in an alien environment that might distort receptor properties. Therefore we have elaborated a new test system which allows studies of individual cytokinin receptors within plant membranes, i.e. more closely to the natural environment. Using this new test system, we have re-evaluated earlier data on ligand-binding properties of some receptors from Arabidopsis (AHK3, AHK2) and maize (ZmHK1). This includes data on ligand association rate, pH-dependence, and ligand specificity of individual receptors within plant membranes. The ligand specificity of receptors toward cytokinin bases was shown to be rather constant irrespective of the test system used. By contrast, cytokinin-9-ribosides displayed much less affinity for receptors in the plant test system as compared to the bacterial one. This agrees with structural data (Hothorn et al., 2011) and indicates that the common transport moiety of cytokinins, zeatin riboside, has by itself no or very weak cytokinin activity. Due to plant test system, the ligand specificity of the full-length Arabidopsis receptor AHK2 was characterized for the first time. The high sensitivity of receptor ZmHK1 to pH led to the suggestion that some cytokinin receptors can play an additional role as a pH sensor in the lumen of the endoplasmic reticulum.

### P3-9 Identification of novel signal molecules and signaling pathways regulating plant development in Arabidopsis by chemical genomics

**Qian Ma[1], Thomas Vain[1], Deepak Kumar Barange[2], Adeline Rigal[1], Fredrik Almqvist[2], Stéphanie Robert[1]**
*[1]Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå Plant Science Center, SE-901 83 Umeå, Sweden; [2]Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden*

E-mail of presenting author: qian.ma@slu.se
E-mail of corresponding author: Stephanie.Robert@slu.se

Plant growth and development require the coordination of individual cell growth within an entire organism. The ubiquitin-proteasome system (UPS) plays an important role in the signaling pathways involved in this process. Due to the functional redundancy of essential genes, the molecular mechanisms of UPS-mediated signaling pathways still remain elusive. In recent years, chemical genomics or the use of small molecules to perturb target protein function, which overcomes the limits caused by gene redundancy or loss-of-function lethality, has emerged as a powerful strategy to study rapid, dynamic and complex biological processes in plants. Following this strategy, we carried out a high-throughput screening of 12560 synthetic compounds from the ChemBridge chemical library on a pair of wild type and UPS-related mutant of *Arabidopsis*. Based on their differential responses, several bioactive molecules regulating specific plant developmental processes were identified and verified to function through UPS by reverse genetic analysis. A forward genetic approach was also applied to characterize the cognate targets and pathways of those compounds, allowing the identification of novel regulatory components in UPS. Furthermore, the biochemical, proteomic and metabolomic analyses of those compounds will aid to decipher their mode of action in depth. We believe that the bioactive compounds characterized in this study can be used as potent chemical tools to dissect UPS-mediated signaling pathways, providing new insights into the molecular mechanisms of UPS regulation of plant growth and development.

### P3-10 Fluorescently labelled cytokinins for in vivo localization of cytokinin-specific binding activities

**Václav Mik[1], Karolina Kubiasová[2], Lenka Zahajská[3], Jaroslav Nisler[1], Ondřej Plíhal[2], Lukáš Spíchal[1], Lucie Plíhalová[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic; [2]Department of Molecular Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic;[3]Isotope Laboratory, Institute of Experimental Botany AS CR, Prague, Czech Republic*

E-mail of presenting author: vaclav.mik@upol.cz

Cytokinins are a group of plant growth regulators that play an important role in plant growth and development throughout the whole life of plants. In presented research we focused on design, synthesis and biological activity of fluorescently labelled derivatives of potent cytokinin, 6-(3-methylbut-2-en-1-ylamino)purine (iP). The iP molecule was labelled with different fluorophores (7-nitro-2,1,3-benzoxadiazole, rhodamine B, dansyl and fluorescein) linked with short alkyl side chain to different positions of purine moiety. The compounds were screened in classical cytokinin bioassays (tobacco callus, *Amaranthus* and detached wheat leaf senescence), as well as for interaction with cytokinin receptors from *A. thaliana* and maize. The most potent compound in cytokinin bioassays was finally used for detection and localization of cytokinin-specific binding activities in *A. thaliana* tissue samples by confocal microscopy.

### P3-11 Computational analysis of Auxin Responsive Elements in *Arabidopsis thaliana* genome

**Victoria Mironova[1], Nadezda Omelyanchuk[1], Victor Levitsky[1]**
*[1]Department of Systems Biology, Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation*

E-mail of presenting author: victoria.v.mironova@gmail.com

In the past 25 years, Auxin Responsive Elements (AuxRE) have been intensely studied, because of their importance and relevance for plant growth and environmental sensing. Nevertheless, only 25 AuxREs in plant promoters have been validated experimentally to date. The fundamental problem of studying auxin signaling pathway is Auxin Responsive Factors (ARFs) are functionally redundant and bind AuxREs in various homo- and hetero- dimer combinations. This is why detecting and validating direct ARF targets and their AuxRE cis-elements is still a challenging task. Plant biologists still recognize AuxRE by TGTCTC consensus, which is broadly distributed in a plant genome. The aim of our study was to fetch additional significant signals for AuxRE and to reveal how they associate with auxin response pattern.
First, we performed sequence analysis of experimentally proven AuxREs. This bioinformatics approach revealed: (1) an extended nucleotide context for AuxREs themselves and (2) coupling motifs, which can be the footprints for ARF dimerization with other transcription factors. Second, we analyzed the distribution of single, multiple and composite AuxREs in *A. thaliana* genome. Third, we performed an intensive testing of all published to date auxin responsive transcriptomes in *A. thaliana.* Genome-wide analysis of associations between the presence of specific AuxRE type in a gene promoter with its auxin responsive expression allowed us to fetch specificity in location, orientation and composite structure of functional AuxREs. Namely, we provide first evidence that direct single, reverse multiple and at least three types of composite AuxRE are enriched around the transcription start site and activate gene expression in auxin response.

### P3-12 Identification of regulators of PIN polarity (repp) mutants in Arabidopsis

**Petra Novakova[1], Maciek Adamowski[1], Jiri Friml[1]**
*[1]Developmental and Cell Biology of Plants, IST Austria, Klosterneuburg, Austria*

E-mail of presenting author: petra.novakova@ist.ac.at

Polarity is a well-established feature of cells important for multiple cellular functions. Proteins are targetted to specific cellular domains at the plasma membrane, in which they are kept by diffusion barriers, such as tight junctions known from animals. In plants, polar domains seem to be defined differently, since no such diffusion barriers could be detected in most cell types. Much of what we know about polarity in plants derives from studying polarly localised plasma membrane proteins of the PIN-formed family (PINs) that are transporters for the plant hormone auxin. A forward genetic screen translates a process of cell polarity at the cellular level into easy-to-score morphological output. The screen is based on the gravitropic root growth in the transgenic *PIN2::PIN1-HA* in *pin2* mutant background, an agravitropic line that shows basal (rootward) mislocalization of PIN1-HA in the root epidermal and cortex cells. The screen has been designed considering the hypothesis that the EMS-induced mutations in putative regulators of PIN polarity will lead to a less polar localization eventually to trigger a switch from basal to apical (shootward) localization of PIN1-HA in the epidermal cells since apical localization is a more „default" stage of polarity. In the light of our promising results, this forward genetic screen will identify polarity components and will give us insight into the mechanism of cell polarity establishment, maintenance and its dynamic regulations in plants.

### P3-13 Patellins: new regulators of PIN polarity during embryo development

**Miriam Palacios[1]**
*[1]Plant Systems Biology, Flanders Institute for Biotechnology, Gent, Belgium*

E-mail of presenting author: mipal@psb.ugent.be

Auxin is a plant-specific signaling molecule that is directly involved in many plant developmental processes. It is transported from cell to cell in a polar manner to generate dynamic gradients that mediate tissue-specific responses. The plant-specific PIN-FORMED proteins (PIN) are largely responsible for this polar auxin transport between cells. PIN proteins mediate auxin efflux and their polar localization within the cell determines the direction of auxin transport. The regulation of PIN localization within the cell depends on the function of so far unknown modulators which are under the transcriptional control of the repressor AXR3. *AXR3*, is defined by three semidominant mutations that result in increased auxin responses. In order to unravel the mechanism of the AXR3-dependent pathway for PIN localization regulation, a microarray was designed to find such genes. A protein subgroup formed by 6 PATELLINS (PATLs) that belong to the phosphatidylinositol transfer proteins (PITP) family was found to be auxin regulated via AXR3. PATLs belong to a protein subfamily characterized by a Sec14-p like domain, suggesting an interesting link between auxin signaling and phosphatidylinositol metabolism regulation. Our data shows that most of the PATLs are co-localizing with PIN proteins at the plasma membrane. Furthermore, multiple combinations of *patl* mutants show strong effects during embryo development, further emphasizing the role of PATLs in auxin-dependent plant development.

## P3-14 Substituted aromatic cytokinin ribosides protect leaves from senescence by keeping the photosystem II active

**Ondřej Plíhal[1], Hana Vylíčilová[2], Alexandra Husičková[3], Jiří Grúz[2], Lukáš Spíchal[2], Lucie Plíhalová[2]**
*[1]Department of Molecular Biology, Palacky University in Olomouc, CRH, Olomouc, Czech Republic; [2]Department of Chemical Biology and Genetics, Palacky University in Olomouc, CRH, Olomouc, Czech Republic; [3]Department of Biophysics, Palacky University in Olomouc, CRH, Olomouc, Czech Republic*

E-mail of presenting author: ondrej.plihal@upol.cz

Purine based cytokinins are important mediators of plant senescence and they are involved in responses to biotic and abiotic stresses. Here, we prepared and tested a number of benzyl positioned $N^6$-(halogenobenzyl) adenosines. The substitutions of chlorine at C2 of the benzyl ring and at C6 position of the adenine molecule were chosen with the respect to the fact that these modifications can improve the anti-stress properties of the parental cytokinin. Synthesized compounds were characterized by available physicochemical methods and screened by cytokinin bioassays of the first choice. Several derivatives showed significant biological activity, mainly in detached wheat leaf senescence bioassays.
Next, we performed a whole-transcript expression analysis of the treated plants to get evidence whether molecular mechanism of action of these derivatives differs from that of unmodified cytokinin base. To this end, two cytokinin derivatives were used to study the anti-stress action of this class of compounds by DNA microarray analysis. Our results confirmed cytokinin properties of the tested compounds as demonstrated by the fact that several type-B response regulators were upregulated. However, in contrast to 6-benzylaminopurine (BAP) treated plants, both cytokinin derivatives caused specific upregulation of several genes related primarily to photosystem II complex, as well as to genes coding for the enzymes involved in photorespiration and metabolism of chlorophyll. Therefore, having those differences in the expression pattern of photosynthetic genes we examined the influence of the cytokinin derivatives and BAP on the function of photosynthetic apparatus. The modified cytokinins significantly slowed down decreases in the chlorophyll content and in the maximum quantum efficiency of PSII photochemistry ($F_V/F_M$) in Arabidopsis. To sum up, the results confirmed our microarray data and further suggested that treatments with selected cytokinin derivatives lead to reprogramming of the gene transcription and specific mechanisms are activated to protect leaves from senescence.

## P3-15 Putative regulators of auxin-dependent PIN repolarization

**Tomáš Prát[1], Wim Grunewald[2], Ricardo Tejos[2], Gergely Molnar[1], Jiří Friml[1]**
*[1]Institute of Science and Technology (IST Austria), Klosterneuburg, Austria; [2]The Flanders Institute for Biotechnology (VIB), Ghent, Belgium*

E-mail of presenting author: tomas.prat@ist.ac.at

In the meristematic region of the root of *Arabidopsis thaliana*, the auxin transporter PIN1 is localized basally (rootward) in the cells of the endodermis, pericycle and vasculature, while PIN2 localize predominantly basally in the young cortex but shows apical (shootward) polarity in epidermal cells. Following treatment by the auxin analog naphtylacetic acid (NAA), the localization of PIN1 changes from basal to inner-lateral in endodermis and pericycle whereas PIN2 in the cortex becomes outer-laterally polarized. Analysis of the auxin-dependent PIN repolarization response in different genetic backgrounds (e.g. $P_{HS}$*:IAA17*$^{axr3\text{-}1}$ and *arf7 arf19*) suggests that this process requires the $SCF^{TIR1}$-Aux/IAA-ARF auxin signaling pathway.
Considering these facts, we performed microarray experiments to reveal yet unknown regulators of PIN repolarization downstream of TIR1 signaling. First, we obtained a dataset of auxin inducible genes in wild type seedlings under our experimental conditions when PIN repolarization occurs. Then, our data were filtered using a second set of microarrays to obtain a list of genes which are originally auxin inducible but show loss of inducibility in the active $P_{HS}$*:IAA17*$^{axr3\text{-}1}$ background. Third, by eliminating known genes involved in auxin homeostasis or signaling and by cross-checking our results with available microarray data from auxin-treated *arf7 arf19* plants, we identified approximately 190 candidates which are potentially novel regulators of auxin-induced PIN repolarization. As a proof of concept, genes of a transcription factor (*WRKY23*) and a phosphatidylinositol kinase (*PIP5K*) were chosen for subsequent analysis and confirmed to be required for auxin-dependent repolarization of the PIN localization.

### P3-16 Cytokinin regulates lateral root spacing in Arabidopsis

**Eswarayya Ramireddy[1], Ling Chang[1], Thomas Schmülling[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: eswar.ramireddy@fu-berlin.de

Root system architecture is a complex trait regulated by intrinsic genetic factors and their interaction with extrinsic environmental cues. Lateral roots form the main part of dicotyledonous plant`s root system and thus play a vital role in shaping root system architecture. The mechanisms that regulate the positioning of lateral roots (LR) along the primary root are still poorly understood. The plant hormone cytokinin plays a vital role as a negative regulator for LR formation and numerous cytokinin metabolism and signaling genes act redundantly during this process. Investigation of mutants and transgenic *Arabidopsis* plants with a lowered cytokinin status suggests that one role of cytokinin is to provide a positional cue controlling spacing of LRs. Roots with a lowered cytokinin status often form LR primordia (LRP) in close proximity which is suppressed in the wild type. We show that specific cytokinin-synthesizing genes of the *IPT* and *LOG* gene families as well as cytokinin signalling genes are required for this suppression. Further we show links between the cytokinin pathway and two other pathways regulating LR spacing. These involve the receptor-like kinase ARABIDOPSIS CRINKLY4 (ACR4) and its putative peptide ligands GLOVEN (GLV) on the one hand and *PLETHORA* genes on the other hand. Together our results indicate that an inhibitory field of cytokinin originating in newly initiated LRP is involved in regulating rhizotactic patterns. Similar to the shoot apical meristem, where auxin- and cytokinin-based morphogenetic gradients are required for achieving spatio-temporal precision during organ formation, LR spacing also depends on mutual crosstalk between cytokinin and other pathways.

### P3-17 Auxin balance in Arabidopsis thaliana mutants, defective in phytohormone transport and signaling

**Daria Romanyuk[1], Vladislav Emelianov[1], Julia Michailova[1], Maria Shishova[1]**
*[1]Department of Plant Physiology and Biochemistry, Saint-Petersburg State University, Saint-Petersburg, Russian Federation*

E-mail of presenting author: daria-rom@yandex.ru

According to recent knowledge, the phytohormone auxin triggers variety of physiological responses, depending on its concentration, number and properties of auxin receptors. Since currently identified mutants with altered auxin sensitivity differ in growth and development from the wild type seedlings, we suppose that biosynthesis, conjugation or transport of phytohormone might be affected. Therefore we analyzed growth, development, auxin concentration and expression of genes, encoding enzymes of auxin biosynthesis, conjugation and transporters in wild type and *aux1-7, axr1-3* and *tir-1* mutants shoots and roots of *A. thaliana* seedlings, grown on medium without or with addition of exogenous auxins (natural auxin IAA – indole-3-acetic acid and its synthetic analogue 1-NAA -1-naphtalenacetic acid). Based on our experimental data we suggest the link between gene expression, auxin concentration and plant development in *A. thaliana* wild type plants and mutants shoots and roots.

### P3-18 Forward genetic screen to dissect PILS5-dependent apical hook and hypocotyl development

**Lin Sun[1], Elena Feraru[1], Jürgen Kleine-Vehn[1]**
*[1]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*

E-mail of presenting author: lin.sun@boku.ac.at
E-mail of corresponding author: juergen.kleine-vehn@boku.ac.at

The phytohormone auxin has a crucial developmental impact and virtually contributes to every aspect of the plant life cycle. The spatiotemporal distribution of auxin depends on a complex interplay between auxin metabolism and intercellular cell-to-cell auxin transport. Recently, reports on putative intracellular auxin carriers, such as PIN-FORMED (PIN) 5/PIN8 and PIN-LIKE (PILS) 2/PILS5, suggest an auxin sequestration mechanism, apparently limiting nuclear auxin signalling. Such a mechanism could possibly enable single cells to individually modulate the retention and availability of auxin. Mutant analyses suggest that intracellular auxin transport influences various developmental aspects.
However, mechanisms that link the activity of PILS presumptive auxin carrier at the endoplasmic reticulum (ER) with auxin metabolism and signalling are not well understood. Given the novel nature of this regulatory mechanism, we have followed an unbiased forward genetic approach to identify molecular players in the PILS5-dependent pathway. *PILS5* gain-of-function affects several aspects of dark grown seedlings, including hypocotyl length and apical hook development. We used an EMS-mutagenized population and have screened for mutants affecting PILS5 activity in dark grown hypocotyls. We selected 28 distinct mutants suppressing or enhancing PILS5 overexpression phenotypes. We are currently mapping 4 PILS5 enhancer mutants and will present the initial characterization of these mutants.

### P3-19 Chemical genomics uncovers a partial agonist of auxin controlling apical hook development

**Thomas Vain[1], Deepak Kumar Barange[2], Qian Ma[1], Per Anders Enquist[2], Sibo Tao[3], Mark Estelle[3], Thomas Moritz[1], Fredrik Almqvist[2], Laurens Pauwels[4], Stéphanie Robert[1]**
*[1]Forest genetics and plant physiology, SLU/Umea Plant Science Center, Umeå, Sweden; [2]Department of Chemistry, Umeå University, Laboratories for Chemical Biology Umeå, Umeå, Sweden; [3]Division of Biological Sciences, Howard Hughes Medical Institute, Estelle Laboratory, University of California San Diego, San Diego, USA; [4]Plant Systems Biology, Ghent University, VIB, Gent, Belgium*

E-mail of presenting author: thomas.vain@slu.se
E-mail of corresponding author: stephanie.robert@slu.se

Auxin is perceived in the nucleus by the $SCF^{TIR1}$ubiquitin E3 ligase complex by increasing the affinity of the TIR1 receptor to the Aux/IAA proteins. Aux/IAA proteins are then ubiquitinated and degraded by the 26S proteasome releasing auxin responsive genes. However, comprehension of auxin signaling is limited by the redundancy, the pleiotropic effects or the essential function of auxin related genes. Here, we report on a chemical genomics screen targeting an upstream regulator of SCF-complexes. It reveals four Developmental Regulators (DR) acting as partial agonists of auxin. Inducing the expression of the auxin responsive synthetic promoter pDR5::GUS in an ectopic manner, these DRs create a large range of phenotypes at the seedling level. Our preliminary *in vitro* results show that the DR4 is able, depending of the TIR1 – Aux/IAA combination, to either promote or inhibit the formation of the co-receptor complex. DR4 abolishes apical hook formation without affecting hypocotyl elongation and is here used to show how auxin perception controls the apical hook development. The apical hook is a developmentally programmed structure formed to protect meristematic cells during seed germination. DR4 structure activity relationship experiments led us to validate the importance of a highly specific structure of the DR4 to achieve its partial auxin action. We used time lapse imaging to show the needs of functional TIR1/AFBs proteins for the DR4 activity. Aux/IAA gain-of-function mutant phenotype corroborate the *in vitro* data letting us identify the TIR1/AFBs – Aux/IAA co-receptor combinations involved during apical hook development. Furthermore pharmacological approaches coupling DR4 and auxinole, an antagonist of auxin, validated the importance of a full auxin perception system to control the sequential phase of differential cell growth during apical hook development. Our set of partial agonists of auxin is opening new ways to understand the control of *Arabidopsis* development regulated by auxin perception.

## P3-20 Synthesis and biological activity of novel aromatic cytokinin ribosides

**Hana Vylicilova[1], Lucie Plihalova[1], Ondřej Plihal[1], Jiri Grúz[2]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic; [2]Laboratory of Growth Regulators, Palacky University, Faculty of Science, Olomouc, Czech Republic*

E-mail of presenting author: hana.vylicilova@upol.cz

Cytokinins (CKs) are plant growth regulators that control many important physiological and developmental processes throughout the whole life of plants. In the presence of another phytohormones, auxin, CKs promote seed germination, stimulate activity of apical meristems, regulate vascular developments and sink/source relationship, and delay leaf senescence. Naturally occurring CKs are adenine derivates substituted at $N^6$ position either by isoprenoid side-chain or aromatic ring.
We have prepared a series of cytokinin derived 2-chloropurineribosides substituted at $N^6$ position by variously halogenated benzyl ring. Prepared compounds were characterized by HPLC-ESI+MS, elemental analysis, and $^1$H and $^{13}$C NMR. Prepared derivatives were tested in a number of cytokinin bioassays (tobacco callus, A*maranthus* and detached wheat leaf senescence), screened for their ability to activate cytokinin receptors and further to inhibit lipid membrane peroxidation.
Generally, prepared compounds showed similar biological activity as a standard (BAP) in tobacco callus and A*maranthus* bioassays. On the other hand, they significantly delayed chlorophyll degradation in detached wheat leaves reaching in several cases even 196% activity of BAP. The most potent compounds were those bearing halogen in *meta* or *para* position of benzyl ring. All prepared derivates were tested in bacteria receptor bioassay, which has been performed for ZmHK1 and ZmHK3 receptors of *Zea mays* and for AHK3 and CRE1/AHK4 from *Arabidopsis thaliana*. At the same time ARR5:GUS cytokinin bioassay was tested by measurement of specific activity -glukuronidase in transgenic *Arabidopsis* seedling. Microarray-based gene expression analysis (Affymetrix) related to genetic senescence network was performed to scatter the information about genes and transcription factors that are influenced by the treatment of the plant with the prepared derivatives.

## P3-21 Characterization of Purine Permeases in Arabidopsis as candidates for cytokinin transport

**Evelyne Zürcher[1], Bruno Müller[1]**
*[1]Institute for Plant Biology University of Zurich, Institute for Plant Biology, Zurich, Switzerland*

E-mail of presenting author: evelyne.zuercher@uzh.ch

Members of the plant-specific purine permease (PUP) family have been implicated in cytokinin transport in yeast complementation studies. We aim to characterize the Arabidopsis PUP members to establish their role in shaping cytokinin signaling landscapes. By in vivo imaging of transgenic Arabidopsis, we have determined subcellular localization of PUPs and their expression patterns. By analysis of plants with deregulated PUP expression levels, we have found that they are involved in maintaining areas of active cytokinin signaling; overexpression of particular members of PUPs leads to ectopic cytokinin responses in the heart-stage embryo, even in the absence of exogenously added cytokinin. Similarily, downregulation of PUP family members by RNAi approaches causes rearrangements in the pattern of cytokinin responsiveness. We conclude that the expression pattern of PUPs contribute to shape the cytokinin signaling landscapes and that PUPs, by means of their transport activity, provide subsets of cells with active cytokinin or precursors thereof to fuel the cytokinin signaling pathway.

### P3-22 Mutants defective in auxin-dependent inhibition of endocytosis – a strive towards understanding auxin efflux control.

**Marta Zwiewka**[1], **Jiří Friml**[2]
[1]*Mendel Centre for Plant Genomics and Proteomics, Central European Institute of Technology (CEITEC) Masaryk University, Brno, Czech Republic;* [2]*Developmental and Cell Biology of Plants, Institute of Science and Technology Austria (IST Austria), Klosterneuburg – Vienna, Austria*

E-mail of presenting author: marta.zwiewka@ceitec.muni.cz
E-mail of corresponding author: Jiri.FRIML@ist.ac.at

Auxin is an important plant hormone and its asymmetric distribution within plant tissues plays pivotal role in growth and development. Those distinct accumulation patterns are maintained by the directional auxin flow between cells that depends on the polar distribution of PIN-FORMED (PIN) auxin efflux carriers on the plasma membrane. PIN activity can be regulated by their membrane abundance and polarity that is controlled by the endocytic trafickcing. PIN recycling from endosomes to the plasma membrane can be inhibited by Brefeldin A (BFA) leading to accumulation of PINs in the so called 'BFA compartments'. Conversely, naturally occurring auxins such as IAA and its synthetic analogue naphthalene-1-acetic acid (NAA) promote the retention of PIN proteins on the plasma membrane also inhibiting their BFA-induced internalization. Notably, the molecular players facilitating the auxin mediated inhibition of endocytosis remain largely unknown. To better understand the mechanisms by which auxin controls its own efflux we performed a forward genetic screen aimed to identify mutants showing defects in the auxin effect on endocytosis. Here we present cellular and seedling phenotypes of mutant plants which are showing defects in auxin-dependent endocytosis inhibition.

# SESSION 4: DEVELOPMENT

## O4-1 Auxin transport and cell polarity in plant development

**Jiří Friml**[1,2]
*[1]Institute of Science and Technology Austria, Klosterneuburg, Austria; [2]Central European Institute of Technology, Masaryk University, Brno, Czech Republic.*

E-mail of presenting author: Jiri.FRIML@ist.ac.at

Plants display adaptive and flexible development that optimally tailors their phenotype to the wide range of environmental conditions. Post-embryonic growth involving the activity of meristems, tissue regeneration, de novo organ formation and tropistic growth responses are unique examples of ways, in which plants shape their form to environmental changes.
Therefore, in plants, more than in other eukaryotes, (re)establishment of cell and tissue polarities along with patterning of different cell types are major developmental themes. The link between cellular polarizing events and macroscopic manifestation of polarity such as specification of different cell types along the axis is provided by the signalling molecule auxin and its polarized transport through tissues. Directional transport between cells underlies many patterning processes, including apical-basal axis formation during embryogenesis, organogenesis, vascular tissue formation and tropisms. Environmental and endogenous signals can be integrated into changes in auxin distribution through their effects on the cellular distribution of PIN auxin transporters. Different PIN proteins, each with specific polar, subcellular localization form a network mediating directional auxin fluxes through different tissues for formation of auxin activity gradients. Within cells, PIN proteins undergo constitutively cycles of a clathrin-dependent endocytosis and recycling to the plasma membrane. Various endogenous and external signals can influence this subcellular dynamics, thus changing polarity of PIN localization and controlling their directional activity. In this view, the PIN-dependent auxin transport network provides one of the key mechanisms underlying the plasticity and adaptability of plant development. We will present latest insights into the feed-back regulation of auxin transport polarity and evolution of both auxin transport and cell polarization mechanisms.

## O4-2 A Molecular Genetic Framework For Protophloem Formation

**Christian S. Hardtke**[1]
*[1]Department of Plant Molecular Biology, University of Lausanne, Biophore Building, Switzerland*

E-mail of presenting author: christian.hardtke@unil.ch

The phloem performs essential systemic functions in tracheophytes, yet little is known about its molecular genetic specification. In the Arabidopsis primary root meristem, the protophloem is the earliest tissue to differentiate and its continuity is essential for post-embryonic meristem growth and thus, root growth. Delicate quantitative interplay between two opposing signaling pathways determines cellular commitment to protophloem sieve element fate, with a recently described protophloem-specific protein acting as a positive, quantitative master regulator. Interference with the specification of protophloem disrupts its continuity through stochastic suppression of protophloem sieve element precursor differentiation and is associated with systemic effects that propagate throughout the meristem, such as globally reduced auxin response. Incidentally, this abolishes the formative sieve element precursor cell division that creates the protophloem and incipient metaphloem cell files. This division apparently depends on, and is particularly sensitive to local auxin activity. Our data thus support a scenario in which the protophloem serves as the principal route for the delivery of essential growth cues that potentiate auxin response in the root meristem, possibly auxin itself.

### O4-3 The molecular basis of gravitropic setpoint angle control

**Stefan Kepinski[1], Suruchi Roychoudhry[2], Peter Grones[3], Jirí Friml[3], Katelyn Sageman[1], Robert Thomas[1], Netta Cohen[4]**
*[1]Centre for Plant Sciences, University of Leeds, Leeds, UK; [2]Centre for Plant Sciences, University of Leeds, Klosterneuburg, Austria; [3]Developmental and Cell Biology of Plants, Institute of Science and Technology Austria, Klosterneuburg, Austria; [4]School of Computing, University of Leeds, Leeds, UK*

E-mail of presenting author: s.kepinski@Leeds.ac.uk

A fundamental feature of plant architecture is that lateral branches are often maintained at specific angles with respect to gravity, a quantity known as the gravitropic setpoint angle (GSA). While the GSA of the primary axis is typically approximately vertical, the GSA values of lateral roots and shoots are most often non-vertical, facilitating the capture of resources both above- and below-ground. Non-vertical GSAs represent an intriguing problem because their maintenance requires corrective growth both with and against the characteristic positive and negative gravitropic responses observed in roots and shoots respectively. Previously we have shown that non-vertical branch GSAs are sustained by means of an antigravitropic offset component acting in tension with gravitropic response to generate stable, gravity-dependent angled growth. This antigravitropic growth component is auxin transport-dependent and is regulated via TIR1/AFB-mediated auxin signalling within the gravity-sensing cells of the root and shoot. Here we will describe our work to understand the molecular events permitting the auxin fluxes driving antigravitropic growth to be generated within the gravity-sensing cell.

### O4-4 Interaction of cytokinin with auxin to control cell elongation and regulate root development

**G. Eric Schaller[1], Ian Street[1],Dennis Mathews[2], Joseph Kieber[1]**
*[1]Department of Biological Sciences, Dartmouth College, Hanover, USA; [2]Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, USA*

E-mail of presenting author: george.e.schaller@dartmouth.edu

Cytokinins inhibit root growth through effects on both cell division and cell elongation. Cytokinin signaling operates via a two-component signaling pathway, culminating in activation of the type-B response regulator transcription factors, *ARR1*, *ARR10*, and *ARR12* being the major contributors to root cytokinin responses in Arabidopsis. We identified the auxin importer AUX1 as a positive regulator of cytokinin responses in the root through a genetic screen. Mutants of *AUX1* affect cytokinin-mediated inhibition of root cell elongation but not of root cell division, suggesting that the primary role of the shootward directed auxin transport mediated by AUX1 is in the control of cell elongation. We identified two mechanisms by which AUX1 positively contributes to the cytokinin response. First, expression of the type-B response regulator *ARR10* is reduced in the *aux1* mutant, an effect predicted to reduce the sensitivity of the root to cytokinin. Consistent with this prediction, transcript levels of cytokinin primary response genes are also reduced in the *aux1* mutant. Second, making use of the *DR5:GFP* auxin reporter, we found that the auxin activity induced in the root epidermal layer by cytokinin is lost in *type-B ARR* and *aux1* mutants, consistent with a shared role in mediating cytokinin-dependent changes in auxin distribution. Analysis of cytokinin effects on *AUX1* expression and an AUX1-YFP reporter support a model in which cytokinin regulates the shootward auxin flux through a localized decrease in AUX1 levels, leading to changes in auxin activity to control cell elongation and ultimately root growth.

### O4-5 Independently formed PLETHORA and auxin gradients orchestrate root growth

**Ari Pekka Mähönen[1], Riccardo Siligato[1], Ondrej Smetana[1], Ben Scheres[2]**
*[1]Institute of Biotechnology, University of Helsinki, Helsinki, Finland; [2]Plant Developmental Biology, Wageningen University Research, Wageningen, The Netherlands*

E-mail of presenting author: AriPekka.Mahonen@helsinki.fi

Plant growth is the outcome of a succession of cytoplasmic growth and cell division, turgor-driven expansion and cell differentiation. In the Arabidopsis root, these processes take place in three anatomically distinct zones: meristem (M), elongation zone (EZ) and differentiation zone (DZ). The phytohormone auxin has been implicated in all the aspects of zonation. In cell suspensions, optimum auxin concentrations are required to maintain cell proliferation and as auxin concentration decreases cells cell can expand and differentiate. However, auxin is also required for cell differentiation, indicating that other factors must select between dividing and differentiating cells. Our new data show that in the root meristem auxin promotes cell division in the presence of PLETHORA (PLT) transcription factors, and in the absence of PLTs, auxin is required for differentiation. Auxin accumulation in the stem cell niche slowly induces PLT transcription but auxin does not dictate the shape of PLT gradient. Instead, our results show that PLT is transcribed in a narrow domain in the proximal meristem from which translated PLT protein uses mitotic segregation and cell-to-cell movement to form a gradient. This new gradient formation mechanism ensures robust dilution of PLT levels along the meristem. When PLT levels are too low to promote cell division, cells proceed with expansion and differentiation. Together our data reveal a dynamic interplay between auxin and PLTs to determine whether cells will remain in mitotic state or whether they enter cell expansion and differentiation, the instrumental processes for organ growth.

### O4-6 Auxin dynamics put polarity in the pod

**Lars Østergaard[1], Joyita Deb[1], Sara Simonini[1], Pauline Stephenson[1], Alejandra Freire-Rios[2], Dolf Weijers[2], Sibu Simon[3], Jiri Friml[3]**
*[1]Department of Crop Genetics, John Innes Centre, Norwich, UK; [2]University of Wageningen, Wageningen, The Netherlands; [3]Institute of Science and Technology, Klosterneuburg, Austria*

E-mail of presenting author: lars.ostergaard@jic.ac.uk

Multicellular organisms including plants and animals develop specialised organs, which are composed of different types of tissues. The structure – or pattern – of organs is determined by the polarity within tissues along axes of symmetry. In order to coordinate polarity across a tissue or organ, multicellular organisms use mobile substances such as hormones.
In plants, auxin plays an essential role in initiating organ formation and in patterning the organs in specific tissue types, including for example lateral roots, young leaves and those of the female reproductive organ, the gynoecium. Auxin signalling is achieved through interactions between the auxin molecule and specific proteins thereby causing the degradation of repressors of gene expression. It has also been established that auxin can influence the direction of its own transport via inhibiting internalisation of PIN auxin transporters.
Interactions among key regulators of *Arabidopsis* gynoecium development have revealed a network of transcription factor activities required for dividing this organ into discrete domains. Regulation of auxin dynamics is emerging as an immediate downstream output from these activities. We show that a set of transcription factors ensure precise auxin distribution to facilitate key events of polarity establishment in gynoecium development. Moreover, recent observations in the lab have revealed a potentially powerful feedback regulatory mechanism for gynoecium development involving a physical interaction between auxin and an ARF-bHLH transcription factor complex. Rather than directing protein degradation, a transcriptomic analysis indicates that this auxin-controlled interaction mechanism determines the identity of downstream targets of the ARF-bHLH complex.
In conclusion, our work is aimed at reaching a mechanistic and developmental understanding of how auxin facilitates precise switches in polarity during plant organ development.

### O4-7 Exploring the role of purine permeases in shaping the cytokinin signaling domains

**Bruno Müller[1], Evelyne Zürcher[1]**
*[1]Institute of Plant Biology, University of Zurich, Zurich, Switzerland*

E-mail of presenting author: bruno.mueller@uzh.ch

The precise localization of cytokinin signaling activities in planta is of vital importance. I will present experiments with Arabidopsis embryos that suggest that cytokinins are actively distributed within the tissue, resulting in defined domains of cytokinin perception. From the large gene family of *purine permeases (PUP)* that code for transmembrane transporters, we have identified specific members that are expressed in correlation to the cytokinin output domains and cause deregulation of cytokinin signaling output upon overexpression and loss of function. These results suggest a model where selected *PUP* genes contribute to define the cytokinin signaling domains in planta.

### O4-8 Specific members of cytokinin, sugar transporter, amino acid permease and cell wall invertase gene families may co-ordinately regulate seed development in forage brassica, B. napus

**Paula Jameson[1], Jiancheng Song[1,2], David O'Keefe[1], Lijun Jiang[2]**
*[1]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; [2]School of Life Sciences, Yantai University, Yantai, China*

E-mail of presenting author: paula.jameson@canterbury.ac.nz

Forage brassica, *B. napus,* is purposely bred for optimised vegetative growth and biomass production. However, the yield of seed remains to be improved for seed producers, but this needs to occur without affecting forage yield and quality. To this end we have targeted the cytokinins, as they are considered to play critical and specific roles during seed development. Recent evidence shows that cytokinins may affect seed yield by enhancing phloem unloading and sugar import into the endosperm through the enzymatic activity of cell wall invertases, sugar transporters and amino acid permeases, and that both seed number and seed size can be affected by manipulation of endogenous cytokinin levels. Our previous work suggests that seed development may be co-ordinately regulated by specific members of cytokinin multi-gene families.
In this work, we isolated most members of cytokinin biosynthesis (*BnIPT1, 2, 3, 5, 7* and *8*) and cytokinin degradation (*BnCKX1* to *BnCKX7*) gene families, as well as cell wall invertase genes (*BnCWINV1* to *BnCWINV4*), sugar transporter genes (*BnSUT1* to *BnSUT4*) and amino acid permease genes (*BnAAP1* to *BnAAP8*), many with both homoeologues from the tetraploid genome. Quantitative expression analysis using RT-qPCR showed temporal and organ-specific expression profiles among members of these multi-gene families in leaves, flowers, pods and seeds. Differential expression patterns of the two homoeologues were also observed for a number of members of each gene family. Specific gene family members and homoeologues have been identified as targets to improve seed yield in this species.

## O4-9 AS2 and BOB1 synergistically regulate cytokinin levels and the establishment of leaf adaxial-abaxial polarity through the ETT/ARF3-IPT3 pathway in Arabidopsis thaliana

**Nanako Ishibashi[1], Yasunori Machida[1], Shoko Kojima[2], Mikiko Kojima[3], Hitoshi Sakakibara[3], Hiro Takahashi[2], Chiyoko Machida[2]**
*[1]Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan; [2]Graduate School of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, A, Japan; [3]Plant Productivity Systems Research Group, RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro, Tsurumi, Yokoh, Japan*

E-mail of presenting author: yas@bio.nagoya-u.ac.jp

The ASYMMETRIC LEAVES2 (AS2) and AS1 of *Arabidopsis* form a complex to act as a repressive epigenetic regulator for the *ETTIN/AUXIN RESPONSE FACTOR3* (*ETT/ARF3*) gene through direct binding to the promoter region, the enhancement of the level of miR390 and DNA methylation in the gene body (Iwasaki et al. *Development* 2013). The BOBBER1 (BOB1), an *Arabidopsis* ortholog of the Nuclear distribution protein C (NudC), plays a pivotal role in the adaxial development of leaves since doubly mutations in *AS2* and *BOB1* genes synergistically cause lack of the adaxial domain of leaves to generate abaxialized filamentous leaves. The lack of the adaxial domain observed in *as2 bob1* is due to enhanced expression of *ETT/ARF3* because a mutation in *ETT* suppresses the formation of filamentous leaves in *as2 bob1* (Ishibashi et al. *Biology Open* 2012). Meta-analyses of microarray of *as2* and *bob1* reveal the enhancement of the level of transcripts of *AtIPT3* gene for cytokinin biosynthetic enzyme, isopentenyl transferase, in shoot apices (Takahashi et al. *Plant Cell Physiol.* 2013). The enhancement of *AtIPT3* levels was abolished by the introduction of mutation in *ETT*, suggesting that *AtIPT3* is positively controlled by *ETT* placed downstream of *AS2* and *BOB1*. Recently, we have determined levels of cytokinin in these mutants. Cytokinin levels increased in the shoot apices of *as2 bob1* as compared to those of the wild type and levels of transcripts of cytokinin-responsive genes, including type-A *Arabidopsis response regulators* (*ARRs*), also increased. In addition, introduction of the *ett* mutation into *as2 bob1* reduced both cytokinin levels and mRNA levels of several type-A *ARR* genes. These results suggest that the control of cytokinin levels in shoot apices and the cytokinin signaling downstream of *ETT/ARF3*, expression of which is regulated by AS2-AS1 and BOB1, are crucial for the development of the adaxial domain of flat leaves.

## O4-10 Hormonal regulation of flower development in the tomato (Solanum lycopersicum L.) mutant stamenless

**Muriel Quinet[1], Gwennaël Bataille[1], Václav Motyka[2], Petre I. Dobrev[2], Stanley Lutts[1]**
*[1]Earth and Life Institute – Agronomy, Université catholique de Louvain, Louvain-la-Neuve, Belgium; [2]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

E-mail of presenting author: muriel.quinet@uclouvain.be

Extensive genetic and molecular studies have led to the broadly accepted ABCE model of flower development. Floral homeotic genes encoding transcription factors combinatorially specify organ identity in each floral whorl. However, less is known regarding how these genes control changes at the cellular level during floral organ formation. Recently, an important role for phytohormones as transducers of genetic information during floral development has been suggested in model species. We investigated the hormonal regulation of floral organ development in a strong *stamenless* (*sl-Pr*) mutant – in the tomato Primabel cultivar – that exhibits homeotic conversion of petals into sepals and stamens into carpels. This phenotype results from mutations in the coding sequence of the class B *Tomato APETALA3* (*TAP3*) gene. Treatments with phytohormones revealed that gibberellins could partly revert the *sl-Pr* phenotype but affected neither the *SL* (*TAP3*) nor the other ABCE gene expression. Quantification of endogenous polyamines and phytohormones content in the *sl-Pr* flowers along their development showed that the *sl-Pr* mutation reduced gibberellins, auxins, cytokinins, salicylic acid, spermine and tyramine concentrations and increased jasmonic acid and abscisic acid in tomato flowers. Together, our results revealed a role of phytohormones in flower development downstream of the *SL* (*TAP3*) gene in tomato. [This research was supported by the Belgian 'Fonds de la Recherche Scientifique' (1.5.101.08.F) and the Czech Science Foundation (506/11/0774)].

## O4-11 Mesoscopic models of auxin-driven morphogenesis in three dimensions

**Przemyslaw Prusinkiewicz[1], Andrew Owens[1], Mik Cieslak[1], Adam Runions[1]**
*[1]Department of Computer Science, University of Calgary, Calgary, CANADA*

E-mail of presenting author: pwp@cpsc.ucalgary.ca

Auxin-driven morphogenesis includes the intertwined processes of: (i) the emergence of points of high auxin concentration characterized by convergent polarization of PIN proteins; (ii) the formation of auxin transport canals (vascular systems and their precursors) that originate at these convergence points; and (iii) the targeting of canals to auxin sinks that may include previously formed vascular strands. Developmentally important examples of auxin-driven morphogenesis include phyllotaxis and the patterning of the form and venation of leaves.

Computational models play an important role in the analysis and understanding of auxin-driven morphogenesis due to its often non-intuitive, self-organizing character. Current models are commonly limited to one or two dimensions and operate at the level of individual cells. However, the understanding of many morphogenetic processes requires computationally efficient three-dimensional models that operate at the level of tissues, organs, or entire plants. Addressing this requirement, we propose mesoscopic models which bridge the microscopic level of individual cells and the macroscopic level of plant architecture. A foundation of these models is a continuous-space mathematical description of polarity and auxin transport, applicable to entire tissues. We apply the resulting techniques to explain vascular patterning in stems, and propose hypothetical models of the vascular patterns that may define the macroscopic architecture of inflorescences. In particular, the models suggest a mechanistic explanation of the development and form of planipaniculate inflorescences, the geometry of which has so far remained elusive. The computational models of inflorescences share many features with the models of phyllotaxis and leaf development, which points to the common geometric logic and molecular implementation of the underlying patterning processes.

## O4-12 Cross-regulatory interactions between florigen, cytokinin and auxin shape shoot architecture and organ morphology in tomato

**Eliezer Lifschitz[1], Yuval Eshed[2], Akiva Shalit-Kaneh[1], Tamar Eviatar-Ribak[1], Tom Laloum[1]**
*[1]Biology, Technion – Israel Institute of Technology, Haifa, Israel; [2]Plant Sciences, Weizmann Institute of Science, Rehovot, Israel*

E-mail of presenting author: lifs@tx.technion.ac.il

Florigen is the newest addition to the list of major growth hormones and the only plant protein with proven long-range regulatory functions. Together with other systemic growth hormones florigen forms a global communication system that informs remote growth zones on overall developmental status.

Grafting experiments established unequivocal one-to-one relations between *SFT*, a tomato homolog of *FT,* and florigen and confirmed quantitative relations between florigen-dependent growth termination and flowering. Further experiments revealed that high *SFT* impacts growth-cessation and termination in all aerial meristems, suggesting thatboosting flowering reflects a fundamental role of florigen as a growth-terminating agent. Growth balance in tomato is checked quantitatively by a meristem specific ratio between *SFT* and its antagonist *SP* whereas the timely switch to termination is regulated by a shift in the SFT/SP ratio stimulated by incoming mobile florigen (Lifschitz et al, 2006, Shalit et al, 2009).

We will show how florigen, in its cell-autonomous and systemic forms, cooperates with cytokinin and auxin to endow each plant axillary meristem with its specific branching potential and developmental fate. We will also describe how new epistatic and synergistic interactions, involving florigen, cytokinin and major meristematic genes such as *WUS* and *KNOX*, control flowering time and organ morphogenesis in tomato. Finally we will unveil the first metabolic ‚end users' of the florigen regulatory system and show how these distinct multi-gene networks are responding, coordinately and quantitatively to changing SFT/SP ratios induced by graft-transmissible florigen.

### O4-13 Cytokinin and the control of shoot branching in Arabidopsis

**Tanya Waldie[1], Dörte Müller[2],Joseph Kieber[3], Ottoline Leyser[1]**
*[1]Sainsbury Laboratory, Cambridge University, Cambridge, UK), [2]Department of Biology, University of York, York, UK), [3]Biology Department, University of North Carolina, Chapel Hill, USA*

E-mail of presenting author: tanya.waldie@slcu.cam.ac.uk

Axillary bud outgrowth is greatly influenced by auxin, cytokinin (CK) and strigolactone. Although the respective inhibitory and stimulatory effects of auxin and CK have been studied for many years, it is still not fully understood how these hormones regulate shoot branching, or how they interact with the more recently-identified strigolactone. In *Arabidopsis*, we have begun to elucidate the role of CK in shoot branching by undertaking mutant analyses with known and newly-identified CK-related genes. A microarray study comparing buds inhibited by apical auxin with those activated by basally-supplied CK has identified several genes that are expressed differentially in response to hormone treatment, with an accompanying loss-of-function branching phenotype. Among these is a specific clade of the type-A *Arabidopsis* Response Regulator (ARR) family, which is CK-up-regulated in buds. This gene family usually negatively regulates CK signalling, but here it was found to be a positive regulator of bud activity, and relevant only in the presence of an intact apex or apical auxin source. Consistent with our type-A ARR analysis, but also counter-intuitive to the general mode of CK signalling is the finding that a member of the type-B positive regulatory family, *arr1*, possesses increased branching. Preliminary studies have shown the type-A and -B mutants possess altered auxin transport, and the increased branching of *arr1* can be rescued by NPA addition, suggesting these CK signalling components may affect auxin transport. We are also interested in understanding the role of CK in modulating the response to nitrate sufficient and insufficient conditions. In the CK biosynthetic triple mutant *ipt3,5,7* and *arr3,4,5,6,7,15* hextuple mutant, the ability to respond to high nitrate conditions and form additional branches is reduced. Our results suggest that these CK genes function to promote branching in the presence of apical auxin, rather than in activating branching following decapitation.

### O4-14 Auxin, self-organization and evolution

**Tom Bennett[1], Sam Brockington[2], Ottoline Leyser[1]**
*[1]Sainsbury Laboratory, University of Cambridge, Cambridge, UK; [2]Department of Plant Sciences, University of Cambridge, Cambridge, UK*

E-mail of presenting author: tom.bennett@slcu.cam.ac.uk

Self-organizing processes, such phyllotaxis and vascular patterning, play a prominent role in plant development. The regulated distribution of auxin by PIN proteins is heavily implicated in many of these phenomena. Furthermore, the self-organization of these processes seems to be driven at least partly by underlying self-organization of PIN protein behaviour. Computational models have demonstrated the plausibility of PIN-driven auxin transport as an explanation for self-organizing systems, but progress in dissecting the cellular mechanisms of PIN protein behaviour that drive these processes has been slow. Here, we discuss the prospects for understanding self-organizing PIN protein behaviours and their evolution.
We have analysed PIN protein behaviour in the shoot, with particular respect to canalization and shoot branching, and demonstrate that different rules of PIN behaviour apply in the shoot and root, and that behaviours also differ amongst PIN proteins in the same cellular context. To understand the contribution of PIN structure to their behaviour, we have undertaken a large-scale structural and phylogenetic re-analysis of PIN proteins, including unprecedented sampling of non-angiosperm PIN genes. We show that PIN protein evolution has generally been very conservative, and major changes in PIN protein structure are unlikely to account for the evolution of different patterns of PIN behaviour. We provide a framework for understanding the evolution of self-organizing developmental processes with respect to land plant evolution, and discuss experimental approaches to dissect the evolution of self-organizing PIN protein behaviour.

### O4-15 Hierarchical self-organizing auxing signaling in Arabidopsis

**Zhenbiao Yang**[1]
*[1]Center for Plant Cell Biology, Institute of Integrated Genome Biology, and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA*

E-mail of presenting author: yang@ucr.edu

We have been using the Arabidopsis leaf pavement cells as a model system to investigate the mechanisms of auxin action. Auxin is required and sufficient for the formation of the pavement cell interdigitation. We recently found that this process is regulated by the cytoplasmic ROP GTPase signaling pathways that require cell surface auxin binding protein 1 (ABP1) and TMK receptor-like kinases. These pathways interact with each other in a self-organizing manner to locally coordinate the formation of interdigitated lobes and indentations within and between pavement cells. In addition, the TIR1-based transcriptional auxin signaling pathway is required for the generation of a global auxin signal that coordinates pavement cell interdigitation throughout the entire leaf epidermis. These findings support the hypothesis that auxin acts as a hierarchical self-organizing signal to regulate the pavement cell interdigitation pattern.

*References:*

1. *Xu et al. 2014. Cell Surface ABP1-TMK auxin sensing complex activates ROP GTPase signaling.* ***Science*** *343:1025-1028.*
2. *Xu et al. 2010. Cell surface- and rho GTPase-based auxin signaling controls cellular interdigitation in Arabidopsis.* ***Cell*** *143:99-110.*

### O4-16 Auxin-dependent differential growth regulation

**Jürgen Kleine-Vehn, Elke Barbez, Chloe Beziat, Kai Dünser, Elena Feraru, Mugurel Feraru, Christian Löfke, Michel Ruiz Rosquete, David Scheuring, Lin Sun**

E-mail of presenting author: juergen.kleine-vehn@boku.ac.at

Multicellular plants require particularly defined cellular strategies for tissue patterning and expansion, because the encapsulating cell wall literally binds neighbouring cells to each other. This interdependency limits cellular migration and, therefore, imposes outstanding importance to cell size determination and supracellular growth regulation. The phytohormone auxin is central to these regulations, but we still lack a comprehensive understanding of how auxin instructs cellular decision and how these responses are coordinated to allow tissue and organ growth.

We are combining cell biological, physiological and developmental genetics approaches to decipher auxin-dependent growth regulation on a sub-cellular (Barbez et al., Nature 2012), tissue (Löfke et al., JIPB 2013) and organ level (Ruiz Rosquete et al., Curr Biol 2013).

On a cellular level auxin activity is controlled on multiple levels, including auxin metabolism and compartmentalization (Barbez and Kleine-Vehn, TIPS 2013). In our lab we unravel the role of the endoplasmic reticulum (ER) in auxin signalling. We are currently dissecting the function of PILS putative auxin carriers at the ER and thereby revealing the developmental importance of ER-based auxin biology. Ultimately, auxin balances cellular division and expansion rates, controlling cell size. Our lab is particularly interested in molecular components that precisely instruct auxin-dependent cell size determination, allowing differential cell size regulation in neighbouring tissues. These levels of regulations jointly allow auxin to control differential tissue expansion, enabling complex responses, such as directional organ growth. To exemplify auxin-dependent organ growth, we study the auxin-dependent control of the root architecture. We currently investigate how auxin steers the radial expansion of root systems, contributing to soil exploration.

Here I will present our novel insights into auxin-dependent differential growth regulation.

### O4-17 Auxin is a central player in the hormone cross-talks that control adventitious rooting

**Catherine Bellini[1], Laurent Gutierrez[2], Gaelle Mongelard[2], Ondrej Novak[3], Mariusz Kowalczyk[4], Daniel Pacular[1], Monica Pacular[1], Irene Perrone[1], Abdellah Lakehal[1], Valerie Legue[5]**
*[1]Plant Physiology – Umea Plant Science Center, Umea University, Umeå, Sweden; [2]Centre de Ressources Régionales en Biologie Moléculaire, Université de Picardie Jules Verne, Amiens, France; [3]Laboratory of Growth Regulators,, Palacký University and Institute of Experimental Botany, Olomouc, Czech Republic; [4]Forest Genetics and Plant Physiology – Umea Plant Science Center, Swedish University of Agricultural Sciences, Umea, Sweden; [5]UMR INRA/Clermont Université 547 PIAF, Université Blaise Pascal, Aubiere, France*

E-mail of presenting author: Catherine.Bellini@umu.se

The plant hormone auxin plays a central role in adventitious rooting and is routinely used to promote adventitious root initiation and development on cuttings of many economically important plant species that are vegetatively propagated.Nevertheless the molecular mechanisms through which it acts only start to emerge. Combining several approaches including a screen for suppressors of the mutation superroot 2, we have identified several genes that are part of a complex regulatory network at the crossroads of auxin, jasmonate or other hormones signaling pathways and controling adventitious root development in Arabidopsis hypocotyl. We'll give an update of the recent advances of our research in Arabidopsis as well as the most recent results obtained with Populus species that, in contrast to Arabisopsis are vegetatively propagated.

### O4-18 Hormonal control of tissue formation in Arabidopsis by integrating growth and patterning

**Bert De Rybel[1], Milad Adibi[2], Alice Sarah Breda[1], Jos Wendrich[1], Ondrej Novak[3], Nobutoshi Yamaguchi[4], Doris Wagner[4], Karin Ljung[4], Christian Fleck[2], Dolf Weijers[1]**
*[1]Biochemistry, Wageningen University (WUR), Wageningen, The Netherlands; [2]Synthetic and Systems Biology, Wageningen University (WUR), Wageningen, The Netherlands; [3]Laboratory of Growth Regulators, Palacky University & Institute of Experimental Botany and Umeå Plant Science Centre, Olomouc, Czech Republic; [4]Department of Biology, University of Pennsylvania, Philadelphia, USA*

E-mail of presenting author: bert.derybel@wur.nl

In all multi-cellular organisms, tissue formation requires a combination of growth and patterning. All vascular root tissues are formed from only four provascular cells early during embryogenesis through oriented cell divisions. We have recently shown that this process is controlled by the auxin-regulated TARGET OF MONOPTEROS 5/ LONESOME HIGHWAY (TMO5/LHW) heterodimer of bHLH transcription factors.
Here, we have identified an enzyme involved in final step of cytokinin biosynthesis, *LONELY GUY4* (*LOG4*), as a direct target gene; suggesting that the TMO5/LHW dimer triggers periclinal cell divisions through local cytokinin activation. However, during growth, the vascular tissues are simultaneously patterned into distinct domains. Using a growing mathematical model and experimental validation, we show that our proposed hormonal interaction network reinforces an early developmental bias in auxin distribution to create a local, non-responding source of the plant hormone cytokinin in the xylem axis. We provide mechanistic and theoretical evidence that these xylem thus act as a local organizer for the entire vascular tissue during embryogenesis by integrating growth via periclinal cell divisions with pattern establishment and maintenance. This non-intuitive genetic network acts as an incoherent feed forward loop in which the intermediates show differential mobility; thus establishing a stable pattern within a growing tissue.

### O4-19 Auxin transport between the shoot stem cell niche and leaf aids in control of organ patterning

**Yuling Jiao[1], Jiyan Qi[1], Ying Wang[2], Ting Yu[1], Alexandre Cunha[3], Teva Vernoux[4], Elliot Meyerowitz[2]**
*[1]State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Beijing, China; [2]Division of Biology and Biological Engineering, Howard Hughes Medical Institute and California Institute of Technology, Pasadena, USA; [3]Center for Advanced Computing Research, Division of Engineering and Applied Science, California Institute of Technology, Pasadena, USA; [4]Laboratoire de Reproduction et Développement des Plantes, CNRS, INRA, ENS Lyon, UCBL, Université de Lyon, Lyon Cedex, France*

E-mail of presenting author: yljiao@genetics.ac.cn

Stem cells are responsible for organogenesis but it is largely unknown if information from a stem cell niche is still required to direct organ patterning. It has long been proposed that the stem cell niche at the plant shoot apex produces a signal that promotes leaf dorsoventral (adaxial-abaxial) patterning. Here we show that an auxin flow from emerging leaf primordia axil to the shoot apical meristem establishes a transient low auxin zone in the boundary-adaxial domain. We demonstrate that both the auxin flow and the low auxin zone are necessary for robust leaf dorsoventral patterning. Furthermore, the auxin signal is mediated by the auxin-responsive transcription factor MONOPTEROS (MP), whose constitutive activation in the adaxial domain promotes abaxial cell fate. Opposite to the original Sussex proposal, instead of a signal derived from the meristem, we show a signaling molecule is departing from the primordium to the meristem to promote leaf patterning.

### O4-20 Bridging the Gap: Graft Formation in Arabidopsis

**Charles Melnyk[1], Elliot Meyerowitz[2]**
*[1]The Sainsbury Laboratory, University of Cambridge, Cambridge, UK; [2]Division of Biology, Caltech, Pasadena, USA*

E-mail of presenting author: cwm26@cam.ac.uk

Plant grafting is an important horticultural technique involving the physical joining of two plants. It has been practiced for thousands of years and today, is commonly used in orchards for dwarfing trees and in vineyards to provide disease resistance. Despite the widespread use of this technique, we know little about the process of graft junction formation at the molecular, developmental or genetic levels. I will present research using *Arabidopsis thaliana* as a model for graft formation. Using fluorescent dyes, hormone-responsive reporters, and cell-identity reporters has allowed a detailed characterization of vascular connection and gene activation. A reverse genetics approach has identified plants unable to graft and uncovered hormone response genes required for graft formation. These results provide a developmental framework for graft formation and point to the critical role of auxin response in this process.

### O4-21 Network topology of the ARF7-ARF19 regulatory network is critical for auxin regulation of transient root growth responses

**Kristine Hill[1], Tatsuaki Goh[1], Nathan Mellor[1], Jaesung Oh[1], Ranjan Swarup[1], Gretchen Hagen[2], Tom Guilfolye[2], Hidehiro Fukaki[3], Malcolm Bennett[1]**
*[1]Centre for Plant Integrative Biology, University of Nottingham, Nottingham, UK; [2]Department of Biochemistry, University of Missouri, Columbia, USA; [3]Graduate School of Science, Kobe University, Kobe, Japan*

E-mail of presenting author: Kristine.Hill@nottingham.ac.uk

Auxin regulates plant development by targeting the degradation of Aux/IAA repressor proteins and de-repressing target genes of the transcription factors termed Auxin Response Factors (ARFs). ARF7 and ARF19 are key regulators of root gravitropism. These genes have been shown to have redundant functions since the *arf7;arf19* double mutant exhibits a severe root gravitropic phenotype, whereas *arf7* or *arf19* single mutants do not. However, the nature of their functional relationship has been unclear to date. We initially report that auxin regulates *ARF19* expression in an ARF7-dependent manner. Next, we perform Chromatin Immuno-Precipitation (ChIP) experiments using antibodies raised against unique domains of ARF7 and ARF19 to demonstrate that ARF7 regulates root gravitropism by directly binding *in vivo* to AuxRE elements within the *ARF19* promoter sequence. Mutating these AuxRE elements disrupts the ability of the *ARF19* genomic sequence to rescue the *arf7;arf19* root gravitropic defect. ChIP-qPCR demonstrates that ARF7 and ARF19 bind promoters of auxin-regulated genes in a sequential manner. Mathematical modelling reveals that this regulatory arrangement provides fine temporal control of auxin responsive gene expression during root gravitropism.

### O4-22 LBD16 and LBD18 Are Linked to Auxin Influx Carriers LAX3 and AUX1 to Control Lateral Root Development in Arabidopsis

**Jungmook Kim[1], Han Woo Lee[1]**
*[1]Bioenergy Science and Technology, Chonnam National University, Gwangju, South Korea*

E-mail of presenting author: jungmkim@jnu.ac.kr

Auxin regulates lateral root (LR) development through a variety of transcriptional regulators including several members of *LBD/ASL* gene family in *Arabidopsis*. Here we demonstrate that *LBD16/ASL18* and *LBD18/ASL20* are linked to auxin influx carriers AUX1 and LAX(Like-AUX1)3 to control LR development in *Arabidopsis*. *lbd16* or *lbd18* mutation did not significantly alter LR emergence decreased by *lax3* mutation, whereas *LBD18* overexpression rescued the defect in LR emergence in *lax3* to the wild-type levels with concomitant overexpression of the *LBD18* target genes, *EXPANSIN14* (*EXP14*) and *EXP17*, and *POLYGALACTURONASE* regulated by *LAX3* with exogenous auxin. Genetic analyses indicated that *AUX1* requires *LBD16* and *LBD18* function for LR initiation and early stages of LR primordium (LRP) development. Analyses of *lbd16 lbd18 lax3 aux1* quadruple mutants implicated *LBD16* and *LBD18* function in LRP development. Expression of LBD18-SRDX in *lbd18* mutant inhibited LR initiation events, periclinal divisions, and LRP development in response to a gravitropic stimulus. LBD18-SRDX in *lbd18* suppressed promoter activities of the cell cycle genes *CDKA1;1* and *CYCB1;1*, whereas LBD18 activated expression of *CDKA1;1* and *CYCB1;1*. Taken together, these results suggest that *LBD18* controls LR emergence downstream of *LAX3* and that *LBD16* and *LBD18* regulate LR initiation and LRP development with *AUX1*.

### O4-23 A link between transcription factor EVERGREEN and auxin in inflorescence development

**Roeska Blankevoort[1], Afke Sietsma[1], Erik Souer[1], Ronald Koes[1]**
*[1]Genetics, VU Amsterdam, Amsterdam, The Netherlands*

E-mail of presenting author: r.k.blankevoort@vu.nl

EVERGREEN (EVG) is a transcription factor of *Petunia hybrida* with a specific role in inflorescence development. Plants with a mutation in *EVG* fail to form flowers. The transition to the flowering phase is not disturbed. However, the floral meristems and sympodial inflorescence meristems (SIMs) fail to separate, resulting in fasciation of the stem and reduced expression of the floral meristem identity (FMI) gene *DOUBLE TOP*. *EVG* mRNA is only expressed in the incipient SIMS and ceases before FMI genes are expressed here. Constitutive expression of EVG causes an overall dwarfism and serration of leaves and increases auxin levels which enables explants to grow independently of auxin on MS media. The same morphological changes were seen in *p35S:GR-EVG* plants upon DEX-induction. One of the proteins that can interact with EVG in yeast cells, EIP112, is a homolog of STY1 in *Arabidopsis.* The downstream targets of this gene, both in *Petunia* as in *Arabidopsis*, are auxin biosynthesis genes of the FLOOZY/YUCCA family. Since auxin is a major player in plant development, it could be possible that EVG functions through auxin. We have transformed *Petunia* plants with the auxin reporter construct *pDR5rev::3xvYFP*, to determine where the auxin maxima are, and whether these are altered in *evg* mutant plants. In wild type *Petunia* plants the auxin maxima occur in similar positions as previously described for the shoot apical meristem of *Arabidopsis*; first auxin establishes the FM, then the different organ primordia within the flower. Also, a qPCR analysis was done on the GR-EVG expressing plants. Surprisingly, the family of major auxin transporters, the PIN proteins, were found to be upregulated upon prolonged DEX induction. Also, some of the genes involved in auxin biosynthesis seem to be (moderately) upregulated. Currently, we are testing the significance of these findings with regard to direct EVG function.

### O4-24 Auxin represses stomatal development in dark-grown seedlings via Aux/IAA proteins

**Martin Balcerowicz[1], Aashish Ranjan[2], Laura Rupprecht[1], Ute Hoecker[1]**
*[1]Botanical Institute, Cluster of Excellence on Plant Sciences, University of Cologne, Cologne, Germany; [2]Department of Plant Biology, UC Davis, Davis, CA, USA*

E-mail of presenting author: m.balcerowicz@uni-koeln.de

Stomata are crucial for the plant's survival as they balance gas exchange and transpiration and thereby strongly affect photosynthetic performance. Thus, their formation is tightly regulated through internal and external factors. Light represents an external factor that strongly promotes stomata formation; hence, mature stomata are found at very low numbers in dark-grown seedlings. We show that auxin-resistant *aux/iaa* mutants such as *axr3-1* exhibit a de-repression of stomata differentiation in dark-grown seedlings. The higher proportion of stomata in the epidermis of dark-grown *axr3-1* mutants when compared to the wild type results from increased cell division in the stomatal lineage. Excessive stomata are also found in dark-grown seedlings of mutants defective in auxin biosynthesis or auxin perception as well as in seedlings treated with the polar auxin transport inhibitor NPA. Consistent with these findings, exogenous auxin can repress stomata formation in light-grown seedlings. Furthermore, genetic analysis revealed that the effect of *axr3-1* on stomatal development requires the bHLH transcription factors SPCH, MUTE and FAMA as well as the YDA MAP kinase cascade, but is independent of the LRR receptor-like protein TMM and the inhibitor of photomorphogenesis COP1. Taken together, these results indicate that auxin signalling contributes to the suppression of stomata differentiation in dark-grown seedlings.

### O4-25 HD-Zip II transcription factors regulate distal stem cell maintenance in the Arabidopsis root

**I. Ruberti[1], L. Turchi[1], V. Ruzza[1], M. Possenti[2], M. Carabelli[1], G. Sessa[1], G. Morelli[2]**
*[1]Institute of Biology, Molecular Medicine and NanoBiotechnology, National Research Council, Rome, Italy; [2]Food and Nutrition Research Centre, Agricultural Research Council, Rome, Italy*

E-mail of presenting author: ida.ruberti@uniroma1.it

Plants' lifelong growth and organ formation are dependent on the activity of pluripotent stem cells. In the root apical meristem (RAM) of *Arabidopsis thaliana*, stem cells surround a small group of less active cells, the Quiescent Center (QC). The QC, together with its surrounding stem cells, forms the root stem cell niche. Recent work has shown that local auxin levels mediate maintenance or differentiation of distal stem cells (DSC) in the root meristem (Ding and Friml, 2010). Auxin signalling for differentiation of DSC requires the IAA17/AXR3, ARF10 and ARF16 transcriptional regulators. The auxin response factors ARF10 and ARF16 repress *WOX5*, a major controller of root stem cell maintenance, and restrict it to the QC, where WOX5 activity is required for the expression of *PLT1*, a master regulator of root development (Ding and Friml, 2010). However, more insights are needed to unravel the network of transcription factors controlling the activity of distal stem cells. Here we show that the ATHB2, ATHB4 and HAT3 HD-Zip II transcription factors – known to play a crucial role in apical embryo development (Turchi et al., 2013) – are required for distal stem cell maintenance in the RAM, as demonstrated by promotion and inhibition of DSC differentiation in the *hat3 athb4 athb2* triple mutant and in the HAT3 overexpressors, respectively. Furthermore, we find that, compared to controls, the *athb2 athb4 hat3* triple loss-of-function mutant displays an increased *DR5rev::GFP* signal in the distal stem cells, whereas it does not show any significant difference in GFP intensity in the QC. Taken together, our data indicate that HD-Zip II proteins contribute to the establishment and/or maintenance of a graded auxin response in the root stem cell niche.

*Ding Z and Friml J. (2010). Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc. Natl. Acad. Sci. USA 107:12046-12051.*
*Turchi L, Carabelli M, Ruzza V, Possenti M, Sassi M, Peñalosa A, Sessa G, Salvi S, Forte V, Morelli G and Ruberti I (2013). Arabidopsis HD-Zip II transcription factors control apical embryo development and meristem function. Development 140:2118-2129.*

### O4-26 Auxin dependent vacuolar biogenesis controls root cell size determination

**C. Löfke[1], J. Kleine-Vehn[1]**
*[1]University of Natural Resources and Life Sciences, Austria*

E-mail of presentig author: christian.loefke@boku.ac.at
E-mail of corresponding author: juergen.kleine-vehn@boku.ac.at

Coordinated cellular development has outstanding importance for tissue growth, ultimately controlling plant architecture. However, cellular growth regulation is poorly understood in plants. Root epidermal cells are regularly spaced in root hair (trichoblast) and non-hair (atrichoblast) cells. The first visible differences between tricho- and atrichoblast cells are already detectable in the meristematic region, where trichoblast cells show a more intense cytoplasmic staining, shorter cells that undergo a higher rate of cell division and a delay in vacuolization relative to cell elongation. We use root epidermal cells as a model system to decipher cell size determination in plants. In this system, we systematically address auxin regulated cellular division, elongation and differentiation events in the root. We particularly focus on auxin-dependent vacuolar biogenesis and its importance in turgor driven cell expansion.

### O4-27 Auxin signaling balances cambium activity in Arabidopsis

**Klaus Brackmann[1], Karin Grünwald[1], Thomas Greb[1]**
*[1]Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria*

E-mail of presenting author: klaus.brackmann@gmi.oeaw.ac.at
E-mail of corresponding author: thomas.greb@gmi.oeaw.ac.at

The vascular cambium is a lateral meristem which harbours an indeterminate group of stem cells and is essential for lateral growth of plant shoot and root axes, for wood formation, and for biomass accumulation. During the initiation of lateral growth in the shoot of the model species *Arabidopsis thaliana*, differentiated cortical cells in interfascicular regions regain stem cell identity and form the interfascicular cambium, which later gives rise to secondary phloem and xylem. The mechanisms underlying this dedifferentiation process and the control of the resulting stem cell niche are poorly understood. Here we identify by transcriptional profiling the auxin-dependent ARF transcription factors ARF3/ETTIN, ARF4, and ARF5/MONOPTEROS to be strongly upregulated during cambium initiation. By analyzing *PROMOTER:YFP* reporter constructs we show that *ARF3* and *ARF4* genes are expressed in broader domains including phloem, xylem and cambium tissues, and that these domains largely overlap with the activity domain of the auxin-response marker *DR5rev:GFP*. In contrast, *ARF5* is expressed specifically in cambium stem cells, indicating a distinct role of ARF5-mediated auxin signaling in cambium regulation. Strikingly, histological analysis of a hypomorphic *arf5* mutant revealed enhanced cambial activity in this background, suggesting a repressive role of ARF5 in cambium regulation. Consistent with this, the expression domain of the cambium marker *PXY:CFP* is enlarged in *arf5* mutants and *ARF5:YFP* activity is initiated rather late during cambium initiation in interfascicular regions. Moreover, positive regulators of cambium activity are repressed upon the pharmacological induction of *ARF5* activity. We are currently in the process of unraveling the role of individual ARFs in cambium regulation by genome-wide analysis of transcriptome remodeling upon the induction of gene activity in different domains of the *Arabidopsis* stem. In summary, we propose that a distinct subset of ARF transcription factors provides a regulatory network balancing stem cell proliferation during later plant growth.

### O4-28 (P4-52) Co-chaperones are involved in auxin controlled root growth in Arabidopsis

**Stefano D'Alessandro[1], Serena Golin[1], Fiorella Lo Schiavo[1], Michela Zottini[1]**
*[1]Department of Biology, University of Padova, PADOVA, Italy*

E-mail of presenting author: stefano.dalessandro.phd@gmail.com

Homologues of the p23 co-chaperone of HSP90 are present in all eukaryotes, suggesting conserved functions for this protein throughout evolution. Although p23 has been deeply studied in animals, little is known about its function in plants.
*Arabidopsis* owns two isoforms of p23 and single knockout mutant lines of the two paralogues show short root phenotype. We demonstrated that the two proteins are both required for root growth, showing no redundancy in this function, and they mainly localize in the root meristem. The impaired root growth of the knockout mutants is linked to an altered auxin homeostasis and rescued by ectopic auxin. Furthermore, a varied auxin distribution was observed in the root of mutants, likely depending on an impaired polar auxin transport. In this direction, PIN proteins resulted down regulated in the mutants and NPA shows lower effects on the root growth of the p23 double knockout.
HSP90 plays a dual role in auxin control, both in auxin distribution and perception, and p23 could modulate auxin distribution through a molecular mechanism involving HSP90 regulation.

## O4-29 Coordinated auxin production and auxin transport orient apical-basal axis in Arabidopsis embryos

**Helene Robert Boisivon[1], Wim Grunewald[2], Chulmin Park[3], Thomas Laux[3], Jiri Friml[4]**
*[1]Mendel Centre for Plant Genomics and Proteomics, CEITEC MU, Brno, Czech Republic; [2]Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB)/Ghent University, Ghent, Belgium; [3]Developmental Biology and Biotechnology of Plants, Institute of Biology III/University of Freiburg, Freiburg, Germany; [4]Developmental and Cell Biology of Plants, Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria*

E-mail of presenting author: helene.robert.boisivon@ceitec.muni.cz

Plant reproduction relies on a well-defined cell division pattern of the fertilized-dependent zygote. The zygote, that becomes embryo, is embedded in maternal-derived integuments, whose influence on embryo development remains elusive. The plant hormone auxin is known to play a crucial role in defining the embryonic body axis. And dynamic transport of the hormone by auxin efflux (PIN proteins) transporters is involved in defining embryonic shoot and root poles. Moreover TAA1/YUCCA-dependent auxin biosynthesis pathway has also an essential function during embryo development, as assayed by the embryonic phenotypes of certain combinations of loss-of-function mutations in these genes. I will present novel data showing the influence of local auxin production, with feedback on auxin transport, at different steps of embryo development. Starting with establishment of the zygote polarity, I will show how fertilization-dependent increase of auxin production in the maternal integuments, and auxin transport from those integuments to the zygote, is important for the first asymmetric division and specification of the apical embryonic pole. At later globular stages, a new auxin source in apical cells of the embryo triggers polarization of the auxin transport to the basal pole for a proper formation of a root. In addition to auxin efflux, a role for auxin influx during embryo development will be discussed. Altogether these data will propose a model integrating the dynamic behavior of auxin production, and its influence on the hormone transport, for the proper development of the embryo.

## O4-30 Auxin does not prepattern floral lateral organ initiation in Arabidopsis

**John Chandler[1], Petra Comelli[1], Dorothea Glowa[1], Wolfgang Werr[1]**
*[1]Institute for Developmental Biology, University of Cologne, Cologne, Germany*

E-mail of presenting author: john.chandler@uni-koeln.de

The first event in *Arabidopsis* floral organogenesis is bract specification, followed by floral meristem (FM) initiation in bract axils, but initiation signals and interplay between both organs remain unelucidated, together with the spatio-temporal patterns of organ initiation in each FM whorl. We have exploited the founder-cell marker *DORNRÖSCHEN-LIKE* (*DRNL*) to analyse patterning and polarity of FM and floral organ initiation with regard to auxin signalling, in wild type and mutants with meristic or positional differences, at a resolution not previously possible.
*DRNL* expression marks incipient bracts at the IM periphery distinct from *DR5* maxima and mutation of core auxin response element motifs within *DRNL* promoter enhancer elements that affect *DRNL* patterning, does not affect *DRNL* expression, suggesting that canonical auxin response is not involved in lateral organ specification. Transcriptome and expression data have shown that the cytokinin response regulator *AHP6*, is putatively directly regulated by *DRNL* in incipient bracts,independent of auxin response, thus, cytokinin signalling might be a bract specifier.
A strong ad-/abaxial polarity drives unidirectional sepal initiation also independently from auxin response patterns. An interplay between bract and sepal founder cell recruitment is shown by *DRNL* expression in *lfy* and *puchi* backgrounds, revealing a bract/leaf-like ground state that questions the identity of incipient floral primordia marked by *DR5*. In *perianthia*, *DRNL* reveals the splitting of sepal founder cell populations, in the absence of a canonical stem-cell population as marked by *CLAVATA3* and *WUSCHEL*, but dependent on *PRESSED FLOWER* and coincident with auxin response, prior to the acquisition of FM autonomy at stage 2 that establishes a centripetal organ initiation mode. For inner whorl organs, auxin response follows stamen initiation but coincides with carpel initiation and late organogenesis steps. Polarity and dynamic founder-cell recruitment represent adaptive traits, but there is little evidence that auxin alone prepatterns floral initiation.

### O4-31 (P4-53) Live mapping the highly dynamic activity patterns of the strigolactone transporter promoter PDR1

**Christian Mattheyer[1], Ernst H. K. Stelzer[1]**
*[1]Physical Biology, Buchmann Institute for Molecular Life Sciences, Frankfurt am Main, Germany*

E-mail presenting author: christian.mattheyer@physikalischebiologie.de

Strigolactones (SL) are secreted by plants into the rhizosphere and were initially characterized as germination stimulants of parasitic weeds [Cook *et al.*, 1966]. However, SL are also required for the establishment of the mycorrhiza symbiosis [Akiyama *et al.*, 2005] and act as phytohormones in plant development [Umeara *et al.*, 2008; Gomez-Roldan *et al.*, 2008]. Biosynthesis of strigolactones is regulated by phosphate availability [López-Ráez *et al.*, 2008] and cross-talks with the phytohormone auxin. Thus, SL integrate external and internal cues and adjust plant growth to nutrient conditions.
The ABC protein PaPDR1 was recently isolated in *Petunia axillaris,* characterized as a cellular SL exporter and proven to be functional also in *Arabidopsis thaliana* [Kretzschmar *et al.*, 2012]. However, the *in planta* routes for SL allocation are not well characterized. We investigate the activity patterns of PaPDR1 and PaPIN1 promoters in *Arabidopsis* and *Petunia* seedlings during germination, main root tip growth and lateral root development to obtain detailed maps of SL transport and their interaction with the transport of auxin. By following the expression of the nuclear-localized pPaPDR1:nls-YFP and pPaPIN1:nls-RFP we discovered that the activity of pPaPDR1 is highly dynamic compared to pPaPIN1. Therefore, we record time lapse images, which exceeding 40h using light sheet-based fluorescence microscopy (LSFM) [Lindek & Stelzer, 1994; Huisken *et al.,* 2004].
High spatio-temporal resolution and low photo toxicity are intrinsic properties of LSFM. They provide a high content analysis of three-dimensional fluorophore distribution in biological specimens as a function of time [Keller & Stelzer, 2008]. Our custom built instruments implement physiological mounting methods, plant illumination between recording intervals and full control of the perfusion media. The close-to-natural conditions ensure the plants vitality during long-term observations [Maizel *et al.*, 2011].

## POSTERS

### P4-1 Influence of purine and phenyl-urea cytokinins on in vitro shoot bud induction from intact seedlings of teak (Tectona grandis L.): a luxurious timber tree of the tropics

**Muhammad Akram[1], Faheem Aftab[1]**
*[1]Department of Botany, University of the Punjab, Lahore-54590, Pakistan, Lahore, Pakistan*

E-mail of presenting author: m.akramks@yahoo.com

Teak (*Tectona grandis* L.) is the most precious hardwood timber-producing trees of the tropics analogous to gold amongst the precious metals. It is used for making fine furniture, shipbuilding, decorative objects and luxurious items. The aim of present investigation was to study the influence of different cytokinins on direct shoot buds induction from intact seedlings and rooting of that shoots. Axenic seedlings around 2 cm long growing on autotrophic MS medium subsequently were cultured on MS medium + 0.08, 0.22, 0.35, 0.80, 2.20 or 3.50 µM of adenine sulphate (ADS), $n^6$-benzyleadenine (BA), kinetin (KIN), thidiazuron (TDZ), and zeatin (ZEA) under *in vitro* conditions for 65 days. Highest (88%) shoot bud induction was obtained at 0.80 µM TDZ with 27.40 mean number and 3.21 cm long shoots. To reduce the residual effect of cytokinins, shoots were cultured onto MS basal medium for 7 days. Such shoots were then rooted on half strength MS medium supplemented with 2, 4, 6 or 8 µM IBA or NAA alone or in combination along with 2 µM putrescine for 25 days. Rooting was highest at 8 + 8 µM IBA + NAA with 3.45 mean number and 4.32 cm long roots. Rooted shoots were then transferred in pots filled with peat moss + soil + sand (2:1:1) under glasshouse conditions and then shifted to the field. We demonstrated that TDZ may be used for direct shoot induction for efficient multiplication of teak.

### P4-2 Exploring the function of cytokinin in regulating flowering time

**Isabel Bartrina[1], Thomas Schmülling[1]**
*[1]Freie Universität Berlin, Germany*

E-mail of presenting author: i.bartrina@fu-berlin.de

Plants undergo a major physiological change as they transition from vegetative to reproductive development. This transition is responsible for the reproductive success of a plant and its timing requires an integration of various endogenous and exogenous signals. Several different genetically defined pathways have been identified to control flowering. The vernalization pathway, the photoperiod pathway, the gibberellin pathway and the autonomous pathway. Most recently, an endogenous pathway that adds plant age to the control of flowering time has been described. The molecular mechanisms of these pathways have been studied extensively in Arabidopsis and several other flowering plants. Although cytokinin has been suspected for long time to be relevant in this developmental transition its role is still unclear.
We have started to compare the flowering time of selected cytokinin metabolism and signalling mutants that have either a higher or lower cytokinin content or signalling activity, respectively. Consistent with the idea that cytokinin plays a promotive role in flowering in Arabidopsis, in particular under non-inductive photoperiods, plants with a higher cytokinin status flower earlier under short-day conditions. In contrast, all mutants having a lower cytokinin status flowered later or were completely unable to flower. To understand the function of cytokinin in the different flowering pathways, we started to characterize the flowering responses of different cytokinin mutants on a molecular level by analysing the expression levels of known regulators of different flowering pathways in these plants after a shift from short- to long-day conditions. Analysis of transcriptomic data and our qRT-PCR analysis indicated a distinct behaviour of known flowering pathway genes dependent of the cytokinin status which might be a cause for the altered flowering behaviour. Hypotheses derived from this analysis are currently being tested in more detail.

### P4-3 Towards understanding how symbiotic signals stimulate lateral root formation in *Medicago truncatula*

**Sandra Bensmihen[1], Violaine Herrbach[1], Céline Remblière[1], Clare Gough[1]**
*[1]LIPM, CNRS, castanet-tolosan, France*

E-mail of presenting author: sandra.bensmihen@toulouse.inra.fr

Legume plants are agronomically and ecologically important because of their capacity to establish two types of endosymbioses: the arbuscular mycorrhizal symbiosis (AMS) with mycorrhizal fungi and the rhizobium-legume symbiosis (RLS) with soil bacteria. These associations provide the plant with a better phosphorus and nitrogen nutrition. Notably, nitrogen fixation during RLS takes place in a new root organ: the nodule, that hosts the bacteria.
Our team demonstrated that both rhizobial and mycorrhizal partners of the legume *Medicago truncatula* produce lipo-chitooligosaccharides (LCOs), called Nod Factors and Myc-LCOs respectively. In addition to their symbiotic roles, those LCOs were shown to stimulate lateral root formation (LRF) in *M. truncatula*. Little is known about the cellular, physiological and genetic mechanisms involved in this LRF stimulation by LCOs. In contrast to *Arabidopsis thaliana*, LRF in *M. truncatula* is not well documented and the comparative ontogeny of LR and nodules is poorly described. Auxin is known as the key hormone of LRF in many species including *M. truncatula.* In addition, auxin is involved in nodule organogenesis, and rhizobial LCOs have been shown to interfere with auxin transport. This suggests that auxin may be an actor in the mechanism of LRF stimulation by LCOs. In this study, we aim to understand the cellular and molecular mechanisms of LRF stimulation by LCOs in *M. truncatula*. We first present a precise description of the successive stages of LRF in this plant using reporter genes and histological sections, and show that LRF in *M. truncatula* differs from the description in *A. thaliana*. Next, we show that LCOs are active on an early stage of LRF. Based on these data, a transcriptomic approach based on auxin and LCO treatments was used to find candidate genes involved in LRF triggered by LCOs. These results will be presented and discussed here.

## P4-4 Light interplay with auxins and cytokinins during tomato fruit ripening

**Ricardo Ernesto Bianchetti[1], Aline Bertinatto Cruz[1], Lazaro Eustaquio Pereira Peres[2], Luciano Freschi[1]**
*[1]Department of Botany, Institute of Bioscience, University of Sao Paulo, Sao Paulo, Brazil; [2]Department of Biological Sciences, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), University of Sao Paulo, Piracicaba, Brazil*

E-mail of presenting author: bianchetti.ricardo@gmail.com

Besides contributing to fruit photosynthesis, light also plays an important role during fruit ripening, acting as a stimulatory signal for processes such as pigment accumulation and plastid biogenesis and differentiation. Not surprisingly, plant hormones are believed to interact with light perception and/or signaling during the regulation of these responses. However, the integration of light and hormone signaling pathways in ripening fruits still remains elusive. In the present study, we investigated fruit ripening in transgenic tomato (*Solanum lycopersicum*) plants carrying synthetic auxin-responsive (*DR5*) or cytokinin-responsive (*ARR5*) promoters fused to the reporter GUS aiming to clarify whether these hormones are also implicated in light-triggered responses in fruit tissues. Greenhouse fruits of these transgenics were harvested at Mature Green stage and incubated under complete darkness or continuous white, red or blue light until achieving 0, 1, 2, 3, 6, 9 and 12 days after Breaker stage. Carotenoid quantification and *in vitro* quantitative assays of GUS activity were performed in pericarp samples collected from these fruits. Both promoters were shown to be significantly more active under white light then under dark conditions. A moderate decrease in the *DR5* activation was observed between the Mature Green and Breaker stages under all light conditions and a progressive increase in this parameter was observed thereafter, being particularly evident in light-exposed fruits. In contrast, *ARR5* activity dramatically decreased soon after the beginning of fruit ripening, achieving minimum values as fast as 3 days after Breaker. Taken together, these results indicate that both auxin and cytokinin activities in fruit tissues are influenced by light during the first ripening stages, but only auxin signaling continues being influenced by light at more advanced ripening phases. The correlation between these hormonal differences and the light impact on fruit carotenoid accumulation will also be presented and discussed (Supported by FAPESP and CNPq).

## P4-5 Transcript profiling of cytokinin-induced cambium activation in Arabidopsis roots

**Tiina Blomster[1], Ari Pekka Mähönen[1]**
*[1]Institute of Biotechnology/Department of Biosciences, University of Helsinki, Helsinki, Finland*

E-mail of presenting author: tiina.blomster@helsinki.fi
E-mail of corresponding author: AriPekka.Mahonen@helsinki.fi

Cambial cell divisions lead to formation of secondary vascular tissues and radial growth of plant organs. In Arabidopsis roots, cambium is established from the procambial tissue soon after the primary development. Plant hormone cytokinin is required for the root secondary growth, but the signalling cascades resulting in cytokinin-induced cambium activation are not well elucidated. The quadruple *isopentenyltransferase (ipt)* mutant *ipt1,3,5,7* defective in cytokinin biosynthesis lacks active root cambium (Matsumoto-Kitano *et al.*, 2008). Accordingly, microarray analysis of one-week-old *ipt1,3,5,7* roots showed decreased expression of genes involved in the cytokinin response, xylem development as well as cambial markers compared to the wild type. The genome-wide transcriptional events during cambium activation of cytokinin-treated *ipt1,3,5,7* roots were studied in a time-course microarray experiment, which yielded abundant changes in gene expression and enriched biological processes. Plant hormone auxin has also been implicated in cambium activation. Prominent rescue of cambium activation was not present after synthetic auxin NAA (1-naphthaleneacetic acid) treatment of *ipt1,3,5,7* roots, but an overlap between transcriptional responses to cytokinin and auxin was observed. Next, the most prominent cambial regulators will be characterized with loss-of-function mutants and expression pattern analysis using histological markers. The results obtained will facilitate understanding of cambium signalling dynamics at the whole-genome level.

*Matsumoto-Kitano et al. (2008) Proc. Natl. Acad. Sci USA 105, 20027–20031*

### P4-6 Functional analysis of auxin- and cytokinin-regulated ERF transcription factor genes in Arabidopsis thaliana

**Sylvia Bolt[1], Ellen Zuther[2], Dirk Hincha[2], Stefanie Zintl[1], Michael Riefler[1], Thomas Schmülling[1]**
*[1]Freie Universität, Institute of Biology/Applied Genetics, Berlin, Germany; [2]Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany*

E-mail of presenting author: sylviabolt@zedat.fu-berlin.de

The Arabidopsisethylene-response factor genes (*ERFs*) form a large gene family encoding plant-specific transcription factors. *ERF*s are involved in regulating numerous developmental processes and are also important for adaption to various biotic and abiotic stresses. Expression of *ERF* genes is regulated by different hormones, including auxin, cytokinin, ethylene and salicylic acid. Here we report the identification of four phylogenetically closely related *ERF* genes from a published microarray study (Brenner et al., 2005) owing to their similar response to cytokinin. Based on phylogenetic analyses of the AP2/ERF-superfamily by Nakano et al. (2006) the genes, named here *ERF-A* to *ERF-D*, are members of the group IX. The objective of this study was to analyze in more detail the transcriptional response of these four genes in response to different cues using quantitative real-time PCR (qRT-PCR), analyze the tissue-specificity of their expression using promoter-GUS fusions, investigate the subcellular localization of ERF-GFP fusion proteins, and finally to study the consequences of loss-of-function as well as gain-of-function mutants. Hormone sensitivity tests indicated that the gene family could be involved in regulating root elongation in response to cytokinin and auxin. Further work investigating the eventual role of *ERF-A* to *ERF-D* in the abiotic stress response point to a particularly relevant function of *ERF-D* in cold stress.

### P4-7 Differential effects of cytokinin and ABA on the growth and development of young rice roots

**Ki Sun Choi[1], Sun Mi Huh[1], Dool Yi Kim[1], Bum Gi Kim[1], Myung Ok Byun[1], Woong June Park[2], In Sun Yoon[1]**
*[1]Molecular Breeding, National Academy of Agricultural Science, Suwon, South Korea; [2]Department of Molecular Biology, Institute of Nanosensor and Biotechnology, Dankook University, Yongin, South Korea*

E-mail of corresponding author: isyoon@korea.kr

The growth of rice root is highly susceptible to environmental stress that involves complex hormonal regulatory networks. In this study, effects of ABA and kinetin on the growth of young rice roots were examined. Both ABA and kinetin inhibit primary root elongation and LR development. However, it is noted that kinetin suppress LRP formation, whereas ABA inhibit LRP emergence, suggesting their differential effects on LR development. A rice TCP promoter-GUS transgenic reporter system was used to monitor auxin level in rice roots, and both kinetin and ABA affect auxin accumulation in LRP. In silico and RT-PCR analysis of gene expression profiling identified rice PIN1 and AUX1 family members regulated by kinetin and ABA treatment, respectively. In addition, we observed a differential effect of kinetin and ABA on the $^{3}$H-IAA accumulation in rice roots. Our data suggests that regulation of auxin transport system is involved, at least in part, in the kinetin effects on the LR development of rice roots.
*Supported by grants (SSAC PJ00951406 and PJ010015) from RDA.*

### P4-8 Regulation of chloroplast development and function: a long-known cytokinin function reassessed

**Cortleven Anne[1], Riefler Michael[1], Schmülling Thomas[1]**
*[1]Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Institute of Biology/Applied Genetics, Berlin, Germany*

E-mail of presenting author: anne.cortleven@fu-berlin.be

A role of the plant hormone cytokinin in regulating the development and activity of chloroplasts was described soon after its discovery. However, most observations reporting this cytokinin function were descriptive and the molecular mechanisms underlying this activity remain elusive despite great progress in understanding the metabolism and cellular signalling of the hormone. The development of chloroplasts from etioplasts is an important event during the dark-to-light transition of seedlings. It is marked by ultrastructural changes of the plastids and coincides with the biosynthesis of chlorophyll.
To elucidate part of the mechanisms behind the role of cytokinin in etioplast-chloroplast transition, the greening rate was evaluated in etiolated seedlings of *Arabidopsis thaliana* which were mutated in the cytokinin receptor genes (*AHK2*, *AHK3* and *CRE1/AHK4*). Results indicated a central role for the cytokinin receptors AHK2 and AHK3 in the greening response. We show that this is due to a strongly reduced and delayed light-responsiveness of the rate-limiting steps of 5-aminolaevulinic acid formation and of the first enzymatic step of the chlorophyll branch during tetrapyrrole biosynthesis. These results demonstrate that a fully functional cytokinin perception is essential for normal chloroplast development during dark-to-light transition. To further reveal the importance of the cytokinin signalling pathway on the etioplast-chloroplast transition, the greening response was evaluated in a set of cytokinin signalling mutants. In addition, because of the known roles of ethylene and gibberellin in chloroplast development, cross-talk between cytokinin and these hormones was examined. These results open the path to obtain a more detailed understanding of the classical cytokinin function in regulating chloroplast development.

### P4-9 Interaction between CUC genes and the auxin-cytokinin network control ovule numbers

**Mara Cucinotta[1], Nadia Elisa Quadrelli[1], Andrea Guazzotti[1], Eva Benkova[2], Candela Cuesta Moliner[2], Lucia Colombo[1]**
*[1]Dipartimento di Bioscienze, Università degli studi di Milano, Milano, Italy; [2]Institute of Science and Technology Austria, Vienna, Austria*

E-mail of presenting author: mara.cucinotta@unimi.it
E-mail of corresponding author: lucia.colombo@unimi.it

The pistil contains ovules that develop into seeds after fertilization. The ovule primordia emerge as lateral organs from a meristematic tissue within the carpel. It is of great importance to understand the mechanisms that control ovule numbers as they ultimately determine the final number of seeds and, thereby, the yield in seed-crop plants.
In the last decades, the fundamental role of plant hormones in ovule initiation has been discovered and few transcription factors have been identified as regulators of ovule number. It is known that PIN-dependent efflux mediates primordium development by supplying auxin to the tip creating an auxin maximum. Cytokinin (CK) also positively regulate ovule primordium formation, indeed plants with higher levels of CK show a large increase in the number of ovules.
Among the transcription factors, CUP SHAPED COTILEDON1 (CUC1) and CUC2 play a crucial role in the determination of ovule number. We found that CUC1 and CUC2 regulate *PIN1* expression and localization in ovule primordia since in the *cuc2 pSTK::RNAi-CUC1, PIN1* was down-regulated and the PIN1 protein was not correctly localized at the cell membrane. Moreover we demonstrated that CK treatment increases the ovule numbers in *cuc2 pSTK::CUC1_RNAi* plants, by acting on the expression and localization of PIN1.
Our main goal is to understand the network controlling ovule initiation. In particular we want to clarify the connection between CUC1, CUC2 and CK in the regulation of the *PIN1* expression needed for primordia formation. Moreover a transcriptome analysis by RNA-deep-sequencing has been performed in order to identify genes that are putatively regulated by CUC1 and CUC2 and that are connected to cytokinin and auxin metabolism and signalling. Some interesting candidate genes have been selected and experiment will be performed to confirm and study their involvement in the molecular and hormonal network responsible for the determination of ovule numbers.

## P4-10 Effects of cytokinins and methyl ester of jasmonic acid on the development of isolated zucchini cotyledons

**Maya Damyanova[1], Dessislava Todorova[1], Zornitsa Katerova[1], Dzhovani Polizoev[1], Iskren Sergiev[1]**
*[1]Regulation of Plant Growth and Development, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria*

E-mail of presenting author: m.damyanova@gmail.com

Isolated zucchini cotyledons were incubated for 6 days at 28°C in darkness or at 16/8h (light/dark) regime on water supplemented with methyl ester of jasmonic acid (MeJA), cytokinins (BA and 4PU-30) or combinations of them. The analyses of catalase (CAT), guaiacol peroxidase (POD), superoxide dismutase (SOD), polyamine oxidase (PAO) and diamine oxidase (DAO) activities, as well as free proline, hydrogen peroxide ($H_2O_2$) and total protein contents were carried out in dynamic from the 2nd day when cell proliferation processes were started till 6th day when any cell division processes were finished. During the experiment, the CAT and SOD activities and protein content were not changed considerably in control and MeJA-treated cotyledons, while POD activity and $H_2O_2$ content were increased significantly. The addition of cytokinins, alone or in combination with MeJA, provoked a further increase of POD activity and $H_2O_2$ content, and the effect of 4PU-30 was more pronounced – up to 6 times as compared to the respective controls. The effect of cytokinins on CAT activity and protein content was the opposite and a significant decrease was detected. Initially, all treatments decreased SOD activity, but afterward it tended to increase. Both cytokinins, applied alone or combined with MeJA, significantly suppressed DAO and PAO activities – up to 4 times (for 4PU-30) as compared to the respective controls. The data about free proline content showed similar trend of alterations. More significant changes in the measured parameters were observed in the cotyledons grown in darkness than those developed in light. These data confirm thatcytokinins and MeJA interact in the regulation of the developmental processes of isolated cotyledons grown under different light regimes.

*Acknowledgments: This work was supported by grant No BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science.*

## P4-11 Novel protein-hormone interactions patterning the gynoecium

**Joyita Deb[1], Sara Simonini[1], Pauline Stephenson[1], Alejandra Freire Rios[3], Dolf Weijers[3], Sibu Simon[4], Jiri Friml[4], Lars Ostergaard[2]**
*[1]John Innes Centre, Norwich, UK; [2]Crop Genetics, John Innes Centre, Norwich, UK; [3]Wageningen University, Wageningen, The Netherlands;[4]Institute of Science and Technology, Klosterneuburg, Austria*

E-mail of presenting author: joyita.deb@jic.ac.uk
E-mail of corresponding author: Lars.Ostergaard@jic.ac.uk

Organ patterning in plants is the output of precise spatio-temporal regulation of gene expression coupled with changes in hormone dynamics. The mediators of these parallel changes are transcription factors and feedback between hormones and these proteins ensures that correct patterning and growth occur simultaneously. Gynoecium development exemplifies the complexity involved in the initiation and establishment of organ patterning owing to the many different domains and structures which comprise the body of this organ. The interplay of transcription factor pathways with the plant hormone auxin within this organ ensures that while auxin initiates gynoecium development, transcription factors impart directionality to the auxin flow and establish correct patterning and organ polarity.

In this project we have found that tissue patterning in the *Arabidopsis* gynoecium proceeds via a novel auxin signalling mechanism, which involves direct interaction between auxin and the transcription factors ETTIN (ETT) and INDEHISCENT (IND). Here we show that both these proteins interact and that auxin is able to bind to this complex. We aim to study whether this signalling module provides auxin feedback control by affecting the polarity of the PIN auxin efflux carriers. We hypothesise that auxin distribution and consequently gynoecium patterning is regulated by this auxin-receptor complex. Furthermore, we have found preliminary evidence that this auxin signalling mechanism is conserved in Brassica spp. indicating that it may have evolutionary significance in organ patterning.

### P4-12 Cytokinins control cell differentiation in woody tissues via transcriptional regulation of NST1 and NST3, the master regulators of secondary cell wall formation in Arabidopsis

**Vojtěch Didi[1], Jana Vašíčková[1], Radek Jupa[2], Mariana Benítez[1,3], Radim Čegan[4], Roman Hobza[4], Shawn Mansfield[5], Vit Gloser[2], Jan Hejátko[1]**

*[1]Functional Genomics and Proteomics of Plants, CEITEC, Masaryk University, Brno, Czech Republic; [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; [3]Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico, Mexico; [4]Department of Plant Developmental Genetics, Institute of Biophysics, The Academy of Sciences of the Czech Republic, Brno, Czech Republic;[5]Department of Wood Science, University of British Columbia, Vancouver, Canada*

E-mail of presenting author: didi@sci.muni.cz
E-mail of corresponding author: hejatko@sci.muni.cz

Control over the equilibrium between cell division and cell differentiation represents one of the core developmental regulations across all kingdoms. Plant hormones cytokinins (CKs) were shown to control the process of cell differentiation in both the shoot and root. However, the molecular mechanism of CK-controlled cell differentiation is far from being understood.

Here we show that CKs act as central regulators of developmental program controlling differentiation of woody tissues including formation of tracheary elements (TEs), the water conducting xylem cells in the Arabidopsis shoot. Our data show that attenuating CK signaling, depleting endogenous CKs or disrupting CK biosynthesis results into formation of decreased number of functional TEs, precocious secondary cell wall formation and increased amount of lignins (especially of acid-insoluble lignins) in the woody tissues of Arabidopsis inflorescence stem. In CK-deficient lines we further observed formation of shortened TEs of smaller diameter, leading to strongly impaired xylem hydraulic conductance. The genome-wide next-gen transcriptional profiling in the CK signaling mutant *ahk2-1 ahk3-1* revealed the role of CK in the regulation of biosynthesis of xylan, lignin and cellulose, the main secondary cell wall compounds. The bioinformatic reconstruction of the upstream regulatory pathway followed by experimental analysis led us to determine the role of CKs in the control over expression of NAC transcription factors *NST1* and *NST3,* themaster regulators of the secondary cell wall formation in Arabidopsis. The resulting model describing novel role of CK in the control of cell differentiation program in woody tissue and its spatiotemporal parameters will be presented.

*Supported by the Czech Science Foundation (13-25280S) and European Regional Development Fund (Central European Institute of Technology project no. CZ.1.05/1.1.00/02.0068).*

### P4-13 Genetic Factors Regulating Branching, Flowering, Leaf Morphology and Ectopic Tuberization in the LOG1 Syndrome of Tomato

**Tamar Eviatar-Ribak[1], Yuval Eshed[2], Eliezer Lifschitz[1]**

*[1]Biology, Technion – Israel Institute of Technology, Haifa, Israel; [2]Plant Sciences, Weizmann Institute of Science, Rehovot, Israel*

E-mail of presenting author: teviatar@tx.technion.ac.il

Plant architecture is shaped by the patterning and fate of its outgrowing axillary meristems. But how apical, lateral and intercalary meristems, all equipped with the same developmental potential, coordinate their homeotic fates with one another is largely unknown. Our approach is to induce heritable developmental syndromes, in which all morphogenetic aberrations share a common cellular mechanism. In practice, auxin or cytokinin (CK) biosynthetic and signaling genes are misexpressed under different promoters and in genetic backgrounds where the basic coordinates of shoot architecture are modified by genes regulating a second growth hormone, primarily florigen. A chosen transgenic syndrome is then subjected to developmental analysis of the relations between the organ-specific aberrations using organ- specific genes.

Using this approach we showed previously how global changes in CK signaling, induced by the tomato ***LOG1*** gene, unleashed hidden potentials of basal axillary meristems of tomato to form homeotic potato-like mini-tubers (Eviatar-Ribak et al, CB 2013). Unlike other major tissues and organs, storage organs in plants vary in their origin, but the regulatory mechanisms underlying their production by a given species or organ remain elusive.

Here we describe new genetic and developmental links between CK signaling, polar auxin transport and meristematic activities uncovered in the novel **LOG1 syndrome.** We show how specific genes regulate activation or suppression of flowering, branching and tuberization in specific axillary meristems along the shoots and in the growing zones along the rachis of compound leaves.

### P4-14 Polarized auxin fluxes in the moss Physcomitrella patens

**Matyas Fendrych[1], Tom Viaene[2], Katarina Landberg[3], Mattias Thelander[3], Eva Medvecka[1], Eric Pederson[3], Elena Feraru[2], Karin Ljung[3], Eva Sundberg[3], Jiri Friml[1]**
*[1]IST Austria, Klosterneuburg, Austria;[2]Plant Systems Biology, VIB, Gent, Belgium; [3]Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, Sweden*

E-mail of presenting author: mates.fendrych@seznam.cz
E-mail of corresponding author: tovia@psb.vib-ugent.be

The emergence and radiation of multicellular land plants was driven by a series of crucial innovations to their body plans. In the advanced land plants, the polarized transport of the phytohormone auxin by PIN auxin efflux carriers asymmetrically localized in the plasma membrane plays a critical role in coordinating the development of multicellular tissues and organs. Here we show that in the moss Physcomitrella patens, asymmetric auxin flux by polarized localization of PIN proteins was operational already in tip-growing cells of the gametophyte. We want to analyze the cellular machinery underlying the PIN polarization in these cells, and we want to use the one-dimensional moss filaments as a model system for cell and auxin flux polarization. The fact that the auxin fluxes were occurring between the individual cells of one dimensional cell files might shed light on the evolution of the auxin flux polarization mechanisms in the more complex tissues of land plants.

### P4-15 Root growth is modulated by differential hormonal sensitivity in neighboring cells

**Yulia Fridman[1], Liron Elkouby[1], Neta Holland[1], Kristina Vragović[1], Rivka Elbaum[2], Sigal Savaldi-Goldstein[1]**
*[1]Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel; [2]Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Rehovot, Israel*

E-mail of presenting author: yuliaf@technion.ac.il
E-mail of corresponding author: sigal@technion.ac.il

Coherent plant growth requires spatial integration of hormonal pathways and cell wall remodeling activities. However, the mechanisms governing sensitivity to hormones and how cell wall structure integrates with hormonal effects are poorly understood. We find that coordination between two types of epidermal root cells, hair- and non-hair cells, establishes root sensitivity to the plant hormones brassinosteroids. While expression of the brassinosteroid receptor BRI1 in hair cells promotes cell elongation in all tissues, its high relative expression in non-hair cells is inhibitory. Elevated ethylene and auxin, and deposition of crystalline cellulose underlie the inhibitory effect of BRI1. We propose that the relative spatial distribution of BRI1, and not its absolute level, fine-tunes growth.

### P4-16 Auxin in rice; leaf shape to yield

**Kenji Fujino**[1]
[1]*Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Sapporo, Japan*

E-mail of presenting author: kfujino@jaffrc.go.jp

In 2050, world population will surpass 9 billion. Plant biology contributes for the satisfy food supply for human demands. One strategy is the development of high yield variety. Therefore, there still remains the understanding of plant architecture for high yield. In last five years, the roles of auxins have been identified in rice, a major crop in the world. Firstly, we identified a gene responsible for leaf shape, narrow leaf, using a mutant line. The *NARROW LEAF 7* encodes auxin biosynthesis gene, a member of *Arabidopsis YUCCA* orthologues. In contrast to *YUCCA*, the single mutant of *NAL7* exhibits its novel phenotype. Then, *NAL1* for polar auxin transport and *NAL2/NAL3* for auxins-transport have been identified as genes for leaf shape using mutant lines. Recently, a naturally occurred mutant allele at *NAL1*, *GPS/SPIKE*, contributes to carboxylation efficiency in photosynthesis rate, resulting high yield. The natural variation of *GPS/SPIKE* was found to be a partial loss-of-function allele of *NAL1*. These clearly show the roles of auxins in rice; from leaf shape to yield.
To enhance the identification of useful genes for high yield potential, we are developing a novel strategy called FATES. The FATES is focusing on the genetic variation among a local population with genetically narrowed, such as elite traits in elite varieties. Now, we try to elucidate genes for high yield potential using a local rice population from a northern-limit of rice cultivation in the world using FATES.

### P4-17 TWISTED DWARF1 regulates auxin transport by interfering with the actin cytoskeleton

**Markus Geisler**[1], **Jinsheng Zhu**[1], **Valpuri Sovero**[2], **Thomas Clement**[3]
[1]*University of Fribourg, Department of Biology – Plant Biology, Fribourg and University of Zurich, Institute of Plant Biology, Zurich, Switzerland;*
[2]*University of Zurich, Institute of Plant Biology, Zurich, Switzerland;*
[3]*CRP-Sante, Oncology Department, Laboratory of Cellular and Molecular Oncology, Luxembourg*

E-mail of presenting author: markus.geisler@unifr.ch

ABCB mediated long-range transport of auxin has been shown to be dependent on physical interaction with the FKBP42, TWISTED DWARF1 (TWD1), documented by a close overlap between twd1 and abcb1 abcb19 dwarf phenotypes. However, the unhanded helical rotation of cell files in twd1 indicates that the actin cytoskeleton may be involved because it is reported that ACTIN7 (ACT7) is responsive to auxin and act7 shows undirected rotated epidermis layers.
Here, we find that *act7* but not *act2* has less branched trichomes, altered auxin-dependent planar root hair polarity similar to twd1. Moreover, confocal imaging shows that *act7* has a lower expression and mislocalized ABCB transporters, which was reported previously for twd1. By analyzing fABD2-GFP as an indicator of actin bundling, we show that both *twd1* and *act7* have reduced actin bundling in the root epidermis and root caps. Additionally, variable angle epifluorescence microscopy (VAEM) imaging also shows lower actin dynamics in twd1. This suggests that either de-regulated actin bundling or mistargeted actin filaments might be the primary cause of the cellular twist in *twd1*. In agreement with published work showing increased NPA binding activity by bundling actin filaments, the bundling of both *act7* and *twd1* hypocotyls is less sensitive toward NPA treatment compared to the wild type.
In summary our data provide evidence that TWD1 might function as an integrator of NPA action effecting actin stability and thus downstream dynamics of auxin efflux transporters.

## P4-18 Defective Protophloem Differentiation Affects Systemically Root Architecture

**Bojan Gujas[1], Antia Rodriguez-Villalon[1], Christian Hardtke[1]**
*[1]Department of Plant Molecular Biology, University of Lausanne, Lausanne, Switzerland*

E-mail of corresponding author: Bojan.Gujas@unil.ch
E-mail of presenting author: christian.hardtke@unil.ch

With increase in size of plants during evolution, efficient transport systems that connect shoot and roots evolved in order to coordinate growth and development. Phloem represents vascular tissue that transports photosynthate, hormones and other growth regulators. It is comprised of conducting tissues (metaphloem and protophloem) and supporting tissue – companion cells. Main phloem transport throughout the root is conducted by metaphloem, formed by interconnected sieve elements tubes that provide efficient passageway only until the root hair zone. Protophloem represents a bridge conducting tissue between root hair zone and division zone of root apical meristems. Protophloem sieve element tubes end deep inside the root meristem, and transport of compounds further towards the root tip depends on less efficient systems, such as transporting proteins or diffusion. Here we show that the peptide ligand CLAVATA3/EMBRYO SURROUNDING REGION 45 (CLE45) specifically inhibits acquisition of protophloem sieve element fate in *Arabidopsis* root meristems, which makes it a tool for investigation of protophloem importance for root development. Similar protophloem differentiation defects in *brevis radix (brx)* are rescued by loss-of-function of a putative CLE45 receptor, BARELY ANY MERISTEM 3 (BAM3), suggesting higher activity of CLE45-BAM3 signaling pathway in *brx* mutants. Inhibition of protophloem differentiation into continuous sieve elements tube has as a consequence lower efficiency of growth regulators, such as auxin, in the meristematic region of the root. This has further indirect implications on formative sieve element precursor cell periclinal divisions that create protophloem and metaphloem cell files. Defects in protophloem differentiation also influence neighboring companion cells differentiation that altogether leads to formation of abnormal phloem poles. The final result of protophloem differentiation disruption is shorter primary root length and a more branched root system, likely due to retention of auxin in differentiated parts of the root where lateral root priming occurs.

## P4-19 The identification of regulators of Arabidopsis primary root vascular development

**Eva Hellmann[1], Hanna Help[1], Sedeer El-Showk[1], Raili Ruonala[1], Ykä Helariutta[1]**
*[1]Institute of Biotechnology / Department of Biological & Environmental Sciences, University of Helsinki, Helsinki, Finland*

E-mail of presenting author: eva.hellmann@helsinki.fi

Vascular tissues enable plants efficient transport of water and nutrients, as well as signaling molecules, metabolites and RNAs, hormones and precursors and provide mechanical strength to the plant body. In the *Arabidopsis* primary root the vasculature consists of a central xylem axis and two poles of phloem, separated by an interveining procambium. Vascular development is, among others, regulated by the interaction of the phytohormones cytokinin and auxin. To analyze their action on plant vascular development, a screen for novel components in primary root vascular development in the cytokinin/auxin context was performed. Hereby EMS mutants showing misexpression of the usually protoxylem- and pericycle-localized *AHP6* were identified in a cytokinin sensitized background or in WT background and await characterization. Interesting mutants show e.g. a reduced *AHP6* expression and cytokinin resistance or asymmetry of *AHP6* expression.

### P4-20 A quest for novel genes and hormonal components regulating Arabidopsis primary root vascular morphogenesis

**Hanna Help-Rinta-Rahko[1], Raili Ruonala[1], Eva Hellmann[1], Ykä Helariutta[1]**
*[1]Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

E-mail of presenting author: hanna.help@helsinki.fi

The *Arabidopsis* primary root vasculature consists of xylem, phloem and intervening pluripotent procambial cells that arise from stem cells within the root apical meristem. The genetic processes that control the formation of these different cell types are of poorly understood. Previously we have shown that both auxin and cytokinin phytohormones are required during root procambial patterning and vascular development in *Arabidopsis* (Mähönen *et al*. Science 2006, Bishopp & Help *et al.* Current Biology 2011, Bishopp *et al.* Current Biology 2011).
In order to find out which other novel components are involved in the abovementioned hormonal interaction controlling vascular patterning I have performed a genetic screen in a cytokinin signalling de-sensitized mutant background. The screen has so far yielded various mutants that show 1) increased sensitivity to ck 2) reduced sensitivity to ck, 3) altered vascular symmetry and 4) gravitropism issues. The response for cytokinin application was reported by observing protoxylem cell identity marker expression patterns, scoring protoxylem differentiation defects, monitoring root elongation and performing histological cross sections to determine the arrangement of vascular cells. The two most interesting mutants analysed show antagonizing phenotypes – one exhibits loss of protoxylem and hypersensitivity to cytokinin in the cre1-12 background – whereas the other shows ectopic protoxylem and little response to cytokinin application, similar to double receptor mutants. The mutant genomes were sequenced for SNPs and both mutations were been mapped to novel loci coding unknown proteins with unknown functions. We are currently characterising the mutants further to reveal their role in vascular patterning. These lines could help us reveal novel components in cytokinin signalling and modulators of auxin domain.

### P4-21 Interaction between cytokinins and TFT proteins during blue light-induced deetiolation of tomato (Solanum lycopersicum L.)

**Petra Hloušková[1], Veronique Bergougnoux[1]**
*[1]Department of Molecular Biology, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacky Univesity in Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: petulkacat@gmail.com
E-mail of corresponding author: veronique.bergougnoux@upol.cz

Light is one of the most important environmental factors influencing plant growth and development all through the plant life cycle. Deetiolation is the first physiological light-regulated event which refers to the switch from skotomorphogenesis to photomorphogenesis. When the etiolated tomato seedling senses the light, the growth rate is rapidly inhibited until it reaches a steady-state level. The blue light (B)-induced deetiolation of tomato is a two-phase process requiring phototropin 1 to induce the fast inhibition occuring within 30 min and cryptochrome 1 to establish the subsequent steady-state growth. We recently identified that during the first phase of deetiolation, free cytokinins (CKs), and more precisely iP, rapidly accumulated in the zone of elongation. Whereas the role of PHOT1 is well investigated in processes such as chloroplast movement or phototropism, the PHOT1-signaling pathway occuring during de-etiolation is not or poorly understood. 14-3-3 proteins, also refered as TFT in tomato, are ubiquitous in eukaryotic cells and function by binding to phosphorylated protein to modulate their activity. They are involved in the wide array of physiological responses, including abiotic and biotic stresses, primary metabolism, growth and cell division, hormone pathways and response to light. Interestingly, some 14-3-3 isoforms of Arabidopsisand barley were found to play a role in de-etiolation. The analysis of the 12 tomato TFTs expression identified that two isoforms are significantly upregulated during de-etiolation: TFT6 and TFT9. TFT9 was found to be specifically upregulated after 1h of exposure to BL indicating that it is not involved in the PHOT1-mediated inhibition of growth but participates in the establishment of the steady-state growth. The *in silico* analysis of its promoter indicated a regulation by CKs. Using a combination of qRT-PCR and luciferase reporter assay, we investigated the CK-regulation of TFT9 expression.

### P4-22 The endogenous ABA promotes hypocotyl growth of etiolated tomato (Solanum lycopersicum L.) seedlings

**Jan Humplík[1], Véronique Bergougnoux[1], Michaela Jandová[2], Jan Šimura[3], Ondřej Novák[4], Aleš Pěnčík[1], Jakub Rolčík[3], Martin Fellner[3]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic; [2]Department of Botany, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic; [3]Laboratory of Growth Regulators, Palacky University in Olomouc and Institute of Experimental Botany, Academy of Science of the Czech Republic, v.v.i, Olomouc, Czech Republic;[4]Department of Metabolomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Academy of Science of the Czech Republic, v.v.i, Olomouc, Czech Republic*

E-mail of presenting author: jan.humplik@upol.cz

The dark-induced growth – skotomorphogenesis is characterized mainly by rapid elongation of hypocotyl. Using the crop model tomato (*Solanum lycopersicum* L.), we studied the role of abscisic acid (ABA) during the development of young tomato seedlings. Our results showed that ABA deficiency led to the inhibition of hypocotyl growth that could be rescued by exogenous ABA. Also, ABA accumulated in dark-grown tomato seedlings characterized by fast growth rate, whereas hypocotyls in blue light-grown seedlings characterized by a slow growth rate accumulated less ABA. Extended elongation during skotomorphogenesis is caused mainly by cell expansion, partly driven by endoreduplication. Here we demonstrated that treatment of ABA-deficient mutant with ABA correlates with up-regulation of *SIKRP1* and *SIKRP3* genes and increased endoreduplication in hypocotyl cells. These data were supported by the analysis of the expression of genes encoding enzymes involved in the ABA metabolism. The present study demonstrates the role of ABA in tomato seedling skotomorphogenesis, and shows more specifically that ABA is important for the process of cell elongation during growth of etiolated seedlings.

### P4-23 Characterization of callus-like tissues in ms07 mutant of Arabidopsis thaliana

**Gyung-Tae Kim[1], Sang Eun Jun[1], Hyun-Sik Yoon[1], Hae In Kwon[1], Thummala Chandrasekhar[2], Jiyoung Hwang[1], Han Sol Kim[1]**
*[1]Department of Molecular Biotechnology, Dong-A University, Busan, South Korea; [2]School of Life Science, Yogi Vemana University, Andhra Pradesh, India*

E-mail of presenting author: kimgt@dau.ac.kr

Differentiated cells of which cell fate was determined for organ and tissue organization undergo dedifferentiation by exogenous supply of auxin and cytokinin, forming indeterminate somatic cell mass, callus. Callus derived by cell dedifferentiation lose cell-type specificity and reacquires totipotency able to regenerate all organs. In spite of repeated study of remarkable ability of cell dedifferentiation in plants, little is known about molecular mechanisms regulating these biological transitions. Here, we identified and characterized *ms07* mutant which forms dedifferentiated (and/or undifferentiated) callus-like tissues. The seedling of *ms07* mutant failed to establish normal shoot and generated undifferentiated cells on the tissues in aerial parts. The supply of the light allowed constant proliferation of greenish callus-like tissues in *ms07* mutant, indicating that photosynthesis was not defective in *ms07* mutant. Interestingly, proliferation of callus-like tissues in *ms07* mutant was maintained without exogenous hormone treatment. The histological analysis showed that the structure of the callus-like cells in *ms07* mutant was similar with that of meristematic cells of wild-type plants. Proliferation of the callus-like tissues in *ms07* mutant was strongly inhibited by high concentration of 2,4-D treatment. Although the treatment of kinetin and NAA to hypocotyls of *ms07* mutant induced callus tissues, normal shoot was never induced. These results suggest that the response to auxin and cytokinin might be disordered in *ms07* mutant. Based on above results, we will discuss about the roles of dedifferentiation process and hormone response during organogenesis.

## P4-24 ASYMMETRIC LEAVES1 (AS1) and AS2 regulate the expression of AtIPT3 through AUXIN RESPONSE FACTOR3 / ETTIN function during leaf development in Arabidopsis thaliana

**Shoko Kojima[1], Hiro Takahashi[2], Nanako Ishibashi[3], Arista Handayani[1], Yoko Matsumura[3], Yasunori Machida[3] and Chiyoko Machida[1]**
*[1]Graduate School of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan; [2]Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo-shi, Chiba 271-8510, Japan; [3] Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan.*

E-mail of presenting author: kojima@isc.chubu.ac.jp

Leaf primordia are generated from the peripheral zone of the shoot apical meristem. In dicot plants, establishment of adaxial-abaxial polarity is required for subsequent development of leaf lamina. The *ASYMMETRIC LEAVES2* (*AS2*) and *AS1* genes of *Arabidopsis thaliana* encode nuclear protein. We showed that AS1-AS2 repress the expression of abaxial identity genes, *AUXIN RESPONSE FACTOR3* (*ARF3*)/*ETTIN* (*ETT*) by directly binding to an upstream region of the ETT genes or by regulating DNA methylation of the *ETT* gene body (Iwasaki et al., 2013). We have identified several genetic enhancers that cause defects in leaf adaxial cell differentiation in the *as1-1* and *as2-1* background. To further elucidate the molecular mechanisms of the AS1/AS2-ETT pathway, we selected two mutations, which seem to affect distinct pathways, the *elongata3* (*elo3*) and the *bobber1* (*bob1*)/*eal* mutations. *ELO3* encodes ELP3, a histone acetyltransferase in the Elongator complex, and *BOB1/EAL* encodes an ortholog of the nuclear movement protein NudC in *Aspergillus nidulans (Kojima et al., 2011, Ishibashi et al., 2012)*. Genetic analysis revealed that *ARF3/ETT* and *ARF4* were responsible for defects of adaxial and abaxial polarity in *as2 elo3* and *as2 bob1* mutant plants. To find key genes downstream of *ARF3/ETT*, we performed a meta-analysis of expression microarray datasets of *as2 elo3* and *as2 bob1* by using the knowledge-based fuzzy adaptive resonance theory (KB-FuzzyART), a clustering algorithm suitable for analysis of expression microarray data. We focused on phytohormone-related genes and found *AtIPT3*, which encodes an adenosine phosphate-isopentenyltransferase (IPT), the enzyme that catalyzes the first step of cytokinin (CK) biosynthesis (Takahashi et al., 2013). We propose that the AS1-AS2-ETT pathway regulates the biosynthesis of CK around the shoot apical meristem in *Arabidopsis thaliana*.

## P4-25 An Improved method for 2-methylthio-cytokinin profiling

**Jakub Kořistka[1], Yu-Qi Feng[2], Petr Tarkowski[1]**
*[1]CRH – Department of Protein Biochemistry and Proteomics, Palacky University, Olomouc, Czech Republic; [2]Department of Chemistry, Wuhan University, Wuhan, China*

E-mail of presenting author: jakub.koristka@gmail.com

2-methylthio-cytokinins are a group of cytokinins (CKs) substituted at the position 2 of the purine ring by methylthio group. These derivates are produced by some microbes, for example by the phytopathogenic actinomycete *Rhodococcus fascians* during the infection. Moreover, 2-MeSCKs have been found in tRNAs that recognize codons beginning with uracil and affect the accuracy of translation. In order to understand cytokinin function in the regulatory pathways that respond to internal and external signals, it is necessary to apply the tools for hormone profiling in plant tissues. LC-MS in combination with solid-pahse extraction offers a powerful tool for rapid profiling of small molecules. The aim of presented study was an improvement of our own SPE-LC-MS method (Tarkowski *et al.,* 2010). The main goal of the work was to develop a faster isolation method and faster chromatographic separation of six 2MeSCKs. Polymer monolith microextraction (PMME), is a simple, solvent free and time-efficient extraction technique. In our research, PMME based on a capillary monolithic column of poly(2-acrylamido-2-methyl-1-propanesulfonic acid-co-ethylene dimethacrylate = poly(AMPS-co-EDMA)) was successfully used for determination of six 2-MeSCKs in bacterial samples. The monolithic material exhibited satisfactory permeability, high mechanical strength and good stability in aqueous solutions. 2MeS CK derivates are captured by this monolith due to its hydrophobic bone with sulfonic groups in the structure which provides strong cation exchange and hydrophobic interactions. Our results show that this method of using monolith poly(AMPS-co-EDMA) is suitable for isolation of 2MeSCK produced by bacteria *Rhodococcus fascians*. New protocol brings 10 times faster sample clean-up and 3 times faster chromatographic separation in comparison with previously published SPE-LC-MS method.

Tarkowski P., Václavíková K., Novák O., Pertry I., Hanuš J., Whenham R., Vereecke D., Šebela M. Strnad M. (2010): Analysis of 2-methylthio-derivates of isoprenoid cytokinins by liquid chromatography-tandem mass spektrometry. *Analytica Chimica Acta*, **680**: 86-91.

### P4-26 Specific Aux/IAA-ARF complexes regulate adventitious root initiation in Arabidopsis

**Abdellah Lakehal[1], Salma Chaabouni[1], Emilie Cavel[1], Ilaria Pigozzo[1], Daniel Ioan Pacurar[1], Irene Perrone[1], Le Hir Rozenn[2], Catherine Bellini[1]**
*[1]Plant Physiology, Umeå Plant Science Centre, Umeå University, SE-90781 Umeå Sweden, Umeå, Sweden; [2]INRA, Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech 78026 Versailles Cedex, France, Versailles, France*

E-mail of presenting author: abdellah.lakehal@umu.se
E-mail of corresponding author: Catherine.Bellini@slu.se

Adventitious roots (AR), also called shoot-born roots, are crucial for vegetative propagation of elite genotypes in horticulture and forestry. AR formation is a plastic polygenic trait controlled by multiple environmental and endogenous factors. The fine-tuning of AR initiation in the *Arabidopsis* hypocotyl is regulated by a complex crosstalk between auxin and jasmonic acid (JA) (1). This signaling pathway include three *AUXIN RESPONSE FACTOR* genes (*ARF6*, *ARF8* and *ARF17*) (2) acting upstream of JA signaling. In this study, we report a genetic analysis which shows that several null allele mutants in the *Aux/IAA* genes developed more AR than the wild type, suggesting that these genes are likely repressing AR initiation through the interaction with ARF6 and ARF8. The analysis by qPCR of the expression profile of these Aux/IAA genes revealed that they are up-regulated in the hypocotyl compared to shoot and roots. In addition, co-immunoprecipitation technique confirmed that at least three of the identified Aux/IAA proteins physically interact with ARF6 and/or ARF8. Our findings highlight novel molecular players in the control of AR initiation in Arabidopsis hypocotyl.
*(1) Gutierrez et al. 2012, Plant cell, 24: 2515-2527. (2) Gutierrez et al. 2009, Plant cell, 21: 3119-3132.*

### P4-27 CFB, a cytokinin-regulated gene encoding a novel F-box protein with multiple functions in development

**Jan Erik Leuendorf[1], Wolfram G. Brenner[1], Thomas Schmülling[1]**
*[1]Institute of Biology – Applied Genetics, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: j.e.leuendorf@fu-berlin.de

The plant hormone cytokinin regulates different aspects in plant development and physiological processes through the transcriptional control of specific genes. In an attempt to identify a core set of cytokinin-regulated genes a meta-analysis of *Arabidopsis* transcriptome studies using CATMA microarrays was performed. One gene, *AT3G44326,* attracted our attention because it ranked second just after the primary cytokinin response gene *ARR6*. The gene encodes a protein containing an F-box domain and was therefore named *CYTOKININ-INDUCED F-BOX* (*CFB*). CFB also contains a predicted $\alpha$-helical transmembrane domain but, interestingly, no other annotated domain of known or unknown function. In *Arabidopsis*, there are two closely related genes, named *CYTOKININ-INDUCED F-BOX-LIKE 1* and *2* (*CFL1* and *CFL2)*. These two genes, however, are not transcriptionally regulated by cytokinin. Whereas single and doubleloss-of-function mutants show no obvious phenotype, the homozygous triple mutant is embryo lethal. Overexpressors of *CFB* show several developmental defects, including a white upper part of the inflorescence stem. The few plastids in the white stem parts are ultrastructurally reminiscent of etioplasts. According to data obtained from GFP fusion proteins, CFB is mainly localized in the cytosol and nucleus, but excluded from the chloroplasts. In interaction studies CFB shows the ability to bind ASK1 in an F-box-dependent manner, which proofs that CFB is part of an SCF E3 ligase in the 26S proteasome degradation pathway. We hypothesize that CFB acts in a cytokinin-regulated pathway influencing the differentiation of chloroplasts by inducing proteolysis of its target proteins.

## P4-28 MAX2 is required for the initial steps of cell division during in vitro shoot regeneration

**Belen Marquez-Garcia[1], Stephen Depuydt[1], Stefaan Werbrouck[2], Danny Vereecke[2], Sofie Goormachtig[1]**
*[1]Department of Plant Systems Biology (VIB) and Department of Plant Biotechnology and Bioinformatics (Ghent University), University of Gent, VIB, Gent, Belgium; [2]Department of Applied Biosciences, University of Gent, Gent, Belgium*

E-mail of presenting author: bemar@psb.vib-ugent.be

To regenerate adventitious shoots in *Arabidopsis thaliana*, root explants are subjected to a two-step process: incubation on auxin rich media (CIM) will lead to dedifferentiation and callus formation while sequential incubation on cytokinin rich media (SIM) will allow shoot induction. Remarkably, *in vitro* shoot regeneration and lateral root formation have common underlying processes and both start with activated divisions of pericycle cells: yet they have different outcomes. The loss of function of *MAX2*, an F-BOX protein involved in the perception of strigolactones leads to a higher lateral root density, however, *max2-1* mutants present problems to regenerate adventitious shoots from root explants. Histochemical staining and qPCR analyses revealed that *MAX2* expression is modulated in a specific spatio-temporal pattern during incubation in CIM and SIM, pointing to its involvement during shoot regeneration. Moreover, microscopical analyses have shown that *max2-1* explants had less cell divisions in the pericycle than wild type, and a lower pCYCB1:GUS expression, already after one day incubation in CIM, pointing that *MAX2* somehow is involved towards the acquiring of competence early on in the process. In addition, a time course experiment at early time points (0, 6, 12 and 24 hours in CIM) revealed an altered expression of auxin and cell cycle related genes. Taken together, our results show that a tight expression of *MAX2* is needed for the initial stages of cell division during the *in vitro* adventitious shoot regeneration process on isolated root explants, with effect in the later stages of the developmental process.

## P4-29 Auxin sensitivities and functions of Aux/IAAs

**Yasushi Mitao[1], Tatsuo Kakimoto[1]**
*[1]Science, Osaka University, Osaka, Japan*

E-mail of presenting author: mitawo94@bio.sci.osaka-u.ac.jp
E-mail of corresponding author: kakimoto@bio.sci.osaka-u.ac.jp

Auxin is involved in regulation of almost all processes of plant growth and development. Different physiological processes are regulated by different ranges of auxin concentrations; however, the underlying mechanisms creating these differences are largely unknown. The first step of auxin signaling is auxin-dependent interaction of an auxin receptor with transcriptional co-repressors (Aux/IAA), which leads to Aux/IAA degradation. *Arabidopsis* has six homologous auxin receptors (TIR1 and five AFBs), 29 Aux/IAA proteins, and two types of active auxins, IAA and phenylacetic acid. Therefore, a large number of possible combinations between these three factors may contribute to the creation of different auxin sensitivities. Firstly, using a yeast heterologous reconstitution system, we investigated auxin-dependent degradation of all *Arabidopsis* Aux/IAAs in combination with every TIR or AFB receptor component. Our results showed that effective auxin concentrations for Aux/IAA degradation depended on both Aux/IAAs and TIR1 or AFB2 receptors, which is consistent with the Aux/IAA-TIR1/AFB co-receptor concept. Secondly, we generated transgenic plants carrying a mutation in the domain II of every *Aux/IAA* to investigate the functions of all *Aux/IAAs*. Phenotypes of reported mutants were reproduced by this transgenic approach. We also identified phenotypes for genes for which mutant phenotypes were unreported. These phenotypes included rootless phenotypes and pin-like phenotypes. This study provides new knowledge of functions of *Aux/IAAs*.

### P4-30 Rheostat control of xylem differentiation by ATHB8/ACL5-BUD2 transcription module

**Giorgio Morelli[1], Simona Baima[1], Marco Possenti[1], Andres Penalosa[1], Guido Leoni[2], Sergio Salvi[1], Barbara Felici[3], Ida Ruberti[4]**
*[1]Food and Nutrition Research Centre CRA-NUT, Agricultural Research Council, Rome, Italy; [2]Department of Physics, Sapienza University of Rome, Rome, Italy; [3]Soil-Plant System Studies Research Centre CRA-RPS, Agricultural Research Council, Rome, Italy; [4]Institute of Molecular Biology and Pathology IBPM, National Research Council, Rome, Italy*

E-mail of presenting author: giorgio.morelli@entecra.it

The role of auxin as main regulator of vascular differentiation is well established, and a direct correlation between the rate of xylem differentiation and the amount of auxin reaching the (pro)cambial cells has been proposed. It has been suggested that thermospermine produced by ACAULIS5 (ACL5) and BUSHY AND DWARF2 (BUD2), is one of the factors downstream to auxin contributing to the regulation of this process in Arabidopsis. Here, we provide an in-depth characterization of the mechanism through which ACL5 modulates xylem differentiation. We show that an increased level of ACL5 slows down xylem differentiation by negatively affecting the expression of *homeodomain-leucine zipper* (*HD-ZIP*) *III* and key auxin signalling genes. This mechanism involves the positive regulation of thermospermine biosynthesis by the HD-ZIP III protein ARABIDOPSIS THALIANA HOMEOBOX8 tightly controlling the expression of *ACL5* and *BUD2*. In addition, we show that the HD-ZIP III protein REVOLUTA contributes to the increased leaf vascularization and long hypocotyl phenotype of *acl5* likely by a direct regulation of auxin signalling genes such as *LIKE AUXIN RESISTANT2* (*LAX2*) and *LAX3*. Through this work we present a model of positive and negative feedback between auxin and HD-ZIP gene expressions that tunes the rate of xylem differentiation.

### P4-31 Shoot organogenesis: linkage and association studies reveal the ABA-responsive gene RPK1 as an essential regeneration factor

**Hans Motte[1], Annelies Vercauteren[1], Stephen Depuydt[1], Danny Geelen[2], Stefaan Werbrouck[3], Sofie Goormachtig[1], Marnick Vuylsteke[1], Danny Vereecke[3]**
*[1]VIB – Plant Systems Biology, Ghent University, Ghent, Belgium; [2]Plant Production, Ghent University, Ghent, Belgium; [3]Applied Biosciences, Ghent University, Ghent, Belgium*

E-mail of presenting author: hamot@psb.ugent.be

*De novo* shoot organogenesis, i.e. the regeneration of shoots on nonmeristematic tissue, is widely applied in plant biotechnology. However, the capacity to regenerate shoots varies highly among plant species and cultivars and the factors underlying regeneration capacity are still poorly understood. Here, we evaluated the shoot regeneration capacity of 88 *Arabidopsis thaliana* accessions and found that the process is blocked at different stages in different accessions. We show that the variation in regeneration capacity between the *Arabidopsis* accessions Nok-3 and Ga-0 is determined by five quantitative trait loci: REG-1 to REG-5. Fine mapping by local association analysis identified the *RECEPTOR-LIKE PROTEIN KINASE1* (*RPK1*) as the most likely gene underlying REG-1. The importance of this abscisic acid-related receptor in shoot regeneration was corroborated with mutant analyses. Altogether our results demonstrate that association mapping in combination with linkage mapping is a powerful method to discover important genes implicated in a biological process as complex as shoot regeneration.

### P4-32 Physiological and phylogenic characterization of *cis*-zeatin-type cytokinins in plants

**Václav Motyka[1], Lenka Záveská Drábková[2], Eva Žižková[1], Petre I. Dobrev[1]**
*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, CZ-165 02 Prague 6, Czech Republic; [2]Department of Taxonomy, Institute of Botany AS CR, CZ-252 43 Průhonice, Czech Republic*

E-mail of presenting author: vmotyka@ueb.cas.cz

Cytokinins (CKs) are a class of phytohormones that regulate various events in plant development. They are structurally diverse and biologically versatile. The most typical representative of isoprenoid CKs in plants is zeatin (Z) that occurs in two isomers, *trans* and *cis*. A majority of biological activity of Z as a free hormone has been for years attributed to *trans*-zeatin (*trans*Z), while *cis*-zeatin (*cis*Z) has been considered as a weakly active CK form with a limited biological relevance.
Despite this viewing we have recently revealed that *cis*Z and its derivatives occur ubiquitously in the plant kingdom and indicated their involvement in control of numerous plant developmental processes and environmental responses (Gajdošová *et al.*, *J Exp Bot 62*: *2827*, *2011*). Our new findings demonstrate a putative role of *cis*Z-type CKs during ontogenesis of higher plants consisting in a delicate regulation of CK levels, especially in states associated with growth-limiting conditions due to internal or external cues (abiotic stress, infection, senescence, seed dormancy) and/or immediately after release from such conditions (early stages of development). Furthermore, a comprehensive screen of over 80 samples across the system of algae, bryophytes and fungi has shown the *cis*Z-type CKs to occur here in high concentrations, in most cases substantially exceeding those of the *trans*Z-types. It suggests that *cis*Z-type CKs are widespread in the evolutionary older organisms being obviously involved in the control of hormonal homeostasis, most probably by substituting a negligible CK-*N*-glucosyltransferase pathway thus fulfilling functions of missing or scarce CK-*N*-glucosides (inactive or poorly active CK forms). Our data imply that the *cis*Z-types have much higher impact for CK biology being more relevant and prevalent in plants than previously supposed.
**Acknowledgements:** *This research was supported by the Czech Science Foundation (506/11/0774).*

### P4-33 Rules of symmetry provides the foundation for organ formation

**Laila Moubayidin[1], Lars Ostergaard[1]**
*[1]Crop Genetics, John Innes Centre, Norwich, UK*

E-mail of presenting author: laila.moubayidin@jic.ac.uk
E-mail of corresponding author: Lars.Ostergaard@jic.ac.uk

In a jigsaw puzzle the final picture emerges from the perfect intersection of individual pieces with only one correct combination. Multicellular organs develop through seemingly complicated interactions between genetic factors and hormone signals. But how this jigsaw of interactive networks ensure the correct outcome in terms of shape and function is unknown. The female reproductive structure in the flower of the *Arabidopsis* model plant, the gynoecium, provides a unique system to study processes of tissue specification and organ growth.
Interactions among key regulators of *Arabidopsis* gynoecium and fruit development have revealed a set of upstream transcription factor activities required to divide the organ into specific domains. Regulation of the plant hormone auxin is emerging as an immediate downstream output from these activities, and here we aim to understand the spatiotemporal information that the interaction between the upstream genetic regulators and auxin provide in the patterning process. Here we show that the transcription factors SPATULA (SPT) and INDEHISENT (IND) control auxin accumulation at the medial-top part of early stages of gynoecium development to achieved symmetry transition and thus allowing the formation of a functionally reproductive structure. SPT and IND repress *PINOID* expression, regulating PIN1 phosphorylation status and its localization at the plasma membrane, thereby ensuring correct tissue polarity and symmetry outcome.
*Arabidopsis* is a close relative of oilseed rape, and by understanding mechanisms that control gynoecium and fruit development in *Arabidopsis*, this project will provide the basis on which more efficient production of oilseed rape can be achieved.

## P4-34 Auxin and cytokinin based markers for the identification of cell divisions in legume nodulation

**Marcin Nadzieja[1], Dugald Reid[1], Jens Stougaard[1]**
*[1]Centre for Carbohydrate Recognition and Signalling, Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*

E-mail of presenting author: marcinjn@mb.au.dk

Legumes are known for their ability to engage in symbiotic relationships with nitrogen-fixing bacteria called rhizobia. This process requires *de novo* establishment of lateral meristems in roots and organogenesis of the bacteria-accommodating structures called nodules. Auxin and cytokinin play essential roles in initiation of the first cortical cell divisions to establish the nodule primordia. Indeed, ectopic application of both hormones has been shown to be sufficient for the establishment of cell divisions and development of nodule like structures in some legumes. Loss-of-function mutants in the Ljlhk cytokinin receptors also showed the neccesity of cytokinin signalling in nodule development. Using auxin and cytokinin specific markers, it is possible to observe the spatio-temporal auxin and cytokinin dynamics during nodule development. We are evaluating which of these markers is most suitable for identifying the earliest possible stages of nodule organogenesis in the model legume *Lotus japonicus*. In this regard, we have evaluated the expression patterns of the Two-Component signalling marker TCSn, the auxin responsive DR5, and the cell division dependent CYCB1;1 markers. With the aid of these markers, we aim to use two complementary techniquues to profile the transcriptome during the first cell divisions of nodule development. We will use both Laser capture microdissection and immunopurification of polysome-mRNA complexes based on nodule primordia specific markers to isolate RNA from these few dividing cells. We anticipate using a reverse-genetics approach making use of the LORE1 insertional mutants in *L. japonicus* to further characterize genes identified through these studies. In parallel with the above, we have used existing whole root and root-window transcriptome data to identify candidate genes associated with cell division for initial screening of LORE1 mutants. Candidates in this approach include for example: cyclins, genes associated with auxin distribution and meristem maintenance and WUSCHEL-related homeobox genes.

## P4-35 Molecular and Physiological Characterization of a Novel mutant allele for IAA9 (entire) In Tomato

**Sapana Nongmaithem[1], Yellamaraju Sreelakshmi[1], Rameshwar Sharma[1]**
*[1]Department of Plant Sciences, University of Hyderabad, Gachibowli, Hyderabad, India*

E-mail of corresponding author: rameshwar.sharma@gmail.com

Plant leaves offer an excellent model to study molecular mechanisms underlying development at cellular and organ level, as they exhibit an enormous degree of phenotypic diversity. Among many genes controlling leaf development, members of the AUX/IAA gene family play a very important role. In forward genetic screening of tomato *cv*., Arka Vikas 120 mM EMS mutagenized population, a mutant with simple leaves (*simple leaf* mutant (*sl*)) phenotype was isolated which was unlike the compound leaf nature of tomato. The *sl* mutant showed a high yield of fruits and interestingly, fruits of *sl* also exhibit temperature dependent parthenocarpy. In order to identify the nature of mutation, we used a candidate gene approach by using CEL-1 enzyme digestion assay which is based on the detection of single nucleotide mismatches in the genomic region of interest. The above assay and the sequencing result of *sl* mutant showed a single base change from G to T, in splice junction site of *IAA9* gene which is different from the single base deletion of *IAA9 entire* mutant. The allelic nature of *sl* and *entire* have been confirmed by allelism test and we are currently examining the role of above novel *IAA9* allele in the regulation of leaf development and parthenocarpy in tomato. Since our mutant has similar phenotype as that of the *IAA9* mutant, it is a promising mutant providing additional allele for *entire* gene.

## P4-36 Interplay between auxin and abscisic acid during the light-induced carbon partition in pseudobulbs and aerial roots of Catasetum fimbriatum.

**Paulo Marcelo Rayner Oliveira[1], Maria Aurineide Rodrigues[1], Gilberto Barbante Kerbauy[1]**
*[1]Department of Botany, University of São Paulo (USP), Institute of Biosciences, Brazil*

E-mail of presenting author: paulorayner@usp.br

*Catasetum fimbriatum* is an epiphytic orchid with a complex and flexible root system. Its absorptive roots usually penetrate through organic substratum nearly cut off from luminosity while the upward-growing roots are exposed to light and can photosynthesize. As for the light-exposed roots, pseudobulbs can also contribute positively to carbon balance by recycling respiratory $CO_2$. In fact, pseudobulbs are essential storage organs for epiphytic orchids and can support growth and metabolic demands of aerial roots lacking light exposure. Light might coordinate shoot and root development by reciprocally regulating auxin gradients between these structures. Besides, the balance between indole-3-acetic acid (IAA) and abscisic acid (ABA) is an important signal for determining the root architecture. This study investigated the potential involvement of auxin and ABA during the light-induced (re)mobilization of carbohydrates in pseudobulbs and aerial roots of *C. fimbriatum*. The source-to-sink relationship between these organs was manipulated by either darkening or exposing the roots to light, while the shoot system were kept fully illuminated for 10 days. Concurrently, treatments with 10µM NPA (N-1-naphthylphthalamic acid), an inhibitor of polar auxin transport, were comparatively studied. The endogenous levels of IAA, ABA, sucrose, glucose, fructose, malate and citrate were analyzed by GC-MS in the youngest pseudobulb and roots. Covering the roots increased the levels of IAA and all carbon sources studied (specially glucose and fructose) in pseudobulbs, while roots showed slightly higher levels of IAA. The concomitant treatment with NPA and lack of light caused a sharp decrease of IAA levels in all organs and an ABA increase in the root system. Interestingly, this last condition induced a conspicuous carbohydrate accumulation in pseudobulbs, with sucrose as the predominant form. A possible interplay between auxin and ABA might participate during these light-controlled responses that influence the root capability of importing carbohydrates from the pseudobulb.
*Supported by CAPES/PROEX.*

## P4-37 The influence of cytokinin on seed germination in Arabidopsis thaliana

**Daniela Pezzetta[1], Stefanie Zintl[1], Jan Erik Leuendorf[1], Michael Riefler[1], Thomas Schmülling[1]**
*[1]Applied Genetics, Freie Universität Berlin, Berlin, Germany*

E-mail of presenting author: dpezzetta@zedat.fu-berlin.de

Seed germination is of crucial importance for a plant's life history as it determines the reproductive success of the progeny generation. Imbibed seeds gather information on their environment in order to initiate germination when certain conditions are met. Besides external cues as light environment, temperature and water availability also internal cues such as genetic parameters, parental nutrition status and phytohormones are known to influence germination. While the role of phytohormones like ABA and GA in germination has been studied extensively, the functional role of cytokinins in germination has been investigated rarely. In our studies we analyzed germination rates of several cytokinin mutants under different light conditions. We found that *Arabidopsis thaliana* seeds with a lowered cytokinin content, which is due to the overexpression of cytokinin oxidase/dehydrogenase genes, and cytokinin receptor mutants showed a dramatically increased light sensitivity in the phyA-mediated 'very low fluence response' (VLFR) as compared to wild type. Cytokinin receptor mutant seeds did not show alterations in GA and/or ABA sensitivity in white light germination assays suggesting that the cytokinin status has no impact on these signalling pathways. Instead, we hypothesize that the cytokinin status modulates light sensitivity and thus seed germination in a more direct fashion. The parental environment is known to influence the germination behaviour of its progeny and it could be that cytokinin plays a role in mediating this environmental effect. The analysis of the mechanism of action of cytokinin in regulating VLFR in seed germination and the eventual relevance of environmental cues is part of our ongoing research.

## P4-38 Auxin and cytokinin derivatives as a new strigolactone mimics

**Tomáš Pospíšil[1], Ondřej Kováč[1], Miroslav Strnad[1]**
*[1]Centre of Region Hana for Biotechnological and Agricultural Research, Palacky University in Olomouc, Olomouc, Czech Republic)*

E-mail of presenting author: t.pospisil@upol.cz

Stigolactones (SLs) are plant hormones with variety of functions. They play key roles in the rhizosphere communication between plants and mycorrhizal fungi; plant roots and shoots development and in a germination of parasitic plant seeds.Since natural SLs have too complex structure for simple routine synthesis the demand for SLs analogues and mimics with simpler structure is enormous. Of course, not only the easier preparations but also very good activity is needed. The additional request from new strigolactone analogues or mimics is a higher stability in the solutions than the natural ones.Our research is focused on the strigolactones as an activator of parasitic plant seeds germination. The design, synthesis and biological activity towards the parasitic seeds germination of novel strigolactone mimics derived from auxins or cytokinines will be presented.

## P4-39 Genome-wide association study identifies novel candidates of hormonal regulation in root development

**Daniela Ristova[1], Wolfgang Busch[1]**
*[1]Busch group, Gregor Mendel Institute for Molecular Plant Biology, Vienna, Austria*

E-mail of presenting author: daniela.ristova@gmi.oeaw.ac.at

Plant hormones play crucial roles in plant development. In particular, auxin and cytokinin regulate many aspects of root development including root polarity, tissue patterning, cell division and cell differentiation. However, many aspects of molecular mechanisms of auxin and cytokinin action, as well as and their interaction in root development, remain to be understood.
Recent reports have shown that genome-wide association (GWA) studies have become powerful tools for the identification of previously uncharacterized genes of basic biological processes. The increasing availability of high-resolution sequence information of many different *Arabidopsis* accessions makes GWA approaches more practicable.
Here we explored the hormonal response of 16 diverse, root growth related traits, quantified by a high-throughput phenotyping pipeline tool developed in our lab, BRAT (Busch Root Analysis Tool). We grew 192 *Arabidopsis* accessions on basal medium for 7 days, followed by transfer to the same media supplemented with auxin (IAA), cytokinin (CK) or both (IAA*CK). Root phenotypes were captured over three consequent days on the hormonal media. GWA study identified about a hundred candidate genes for each hormonal treatment, when we consider 5000 bp surrounding the SNP position. We identified several genes already implicated in root development, for example *AIR1* (*Auxin-Induced in Root cultures 1*), which was significantly associated with two root traits. However, most of the significant GWAS hits were in proximity of novel candidate genes implicated in a broad array of biological processes, including regulation of transcription, protein phosphorylation, oxidation-reduction processes, carbohydrate metabolic processes, circadian rhythm. We are currently selecting the most meaningful candidate genes, based on the sequence polymorphism, p-values of associations and their associated phenotypic traits. We will test the phenotypic consequences in loss of function lines and conduct detailed gene characterization with regard to their involvement in root development.

## P4-40 Auxin modulates vacuolar morphology in an actin-dependent manner

**David Scheuring[1], Jürgen Kleine-Vehn[1]**
*[1]University of Natural Resources and Life Sciences, Austria*

E-mail of presenting author: david.scheuring@boku.ac.at

Function and orientation of the cytoskeleton are crucial for a wide range of processes, including cell division and growth (*1*). Several phytohormons (e.g. auxin and brassinosteroids), which regulate plant development and growth, have been shown to directly act on the organization of the cytoskeleton (*2*). However, mechanistic importance for plant development largely remains to be illustrated.
Here we show that auxin modulates cytoskeletal activity to affect vacuolar morphology, which is required for meristematic cell size determination. We have used pharmacological and genetic approaches to decipher the impact of auxin on the actin cytoskeleton and its consequences for vacuolar structure. Our data suggest that myosin motor proteins along actin filaments provide forces to regulate vacuolar morphology. This modulatory role is required for the auxin effect on meristematic cell size determination and root organ growth.

1. *T. D. Pollard, J. A. Cooper, Actin, a central player in cell shape and movement.* Science ***326**, 1208 (Nov 27, 2009).*
2. *M. Lanza* et al., *Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants.* Developmental cell ***22**, 1275 (Jun 12, 2012).*

## P4-41 Overexpression of the cytosolic cytokinin oxidase/dehydrogenase (CKX7) from Arabidopsis causes specific changes in root growth and xylem differentiation

**Ireen Schwarz[1], Ondřej Novák[2], Miroslav Strnad[2], Thomas Schmülling[1], Tomáš Werner[1]**
*[1]Institute of Biology – Applied Genetics, Freie Universität Berlin – Dahlem Centre of Plant Sciences, Berlin, Germany; [2]Laboratory of Growth Regulators, Palacký University – Institute of Experimental Botany, Olomouc, Czech Republic*

E-mail of presenting author: ireen.schwarz@fu-berlin.de

Degradation of the plant hormone cytokinin is catalyzed by cytokinin oxidase/dehydrogenase (CKX) enzymes encoded by seven genes in *Arabidopsis*. Individual CKX proteins differ in subcellular localization and substrate specificity. We show that *CKX7:GUS* is expressed in the vasculature, the transmitting tissue and the mature embryo sac. A CKX7-GFP fusion protein localized to the cytosol which is unique among all CKX family members. *35S:CKX7*-expressing plants developed short, early-terminating primary roots with vascular bundles containing only protoxylem elements, thus resembling the *wol* mutant of the *CRE1/AHK4* receptor gene. We could show that CRE1/AHK4 activity is required to establish the *CKX7* overexpression phenotype. Several cytokinin metabolites, in particular *cis*-zeatin (*cZ*) and *N*-glucoside cytokinins, were depleted stronger in *35S:CKX7* plants compared to plants overexpressing other *CKX* genes. Interestingly, enhanced protoxylem formation together with reduced primary root growth was also found in the *cZ*-deficient tRNA isopentenyltransferase mutant *ipt2,9*, However, different cytokinins were similarly efficient in suppressing *35S:CKX7* and *ipt2,9* vascular phenotypes. Therefore, we hypothesize that the pool of cytosolic cytokinins is particularly relevant in the root procambium where it mediates vascular tissue differentiation through CRE1/AHK4. Taken together, the distinct consequences of *CKX7* overexpression indicate that the cellular compartmentation of cytokinin degradation and substrate preference of CKX isoforms are relevant parameters defining the activities of the hormone.

### P4-42 ETTIN/ARF3 interacts with tissue-specific transcription factors to mediate its function

**Sara Simonini[1], Lars Ostergaard[1]**
*[1]Crop Genetics, John Innes Centre, Norwich, UK*

E-mail of presenting author: sara.simonini@jic.ac.uk

The formation of organs within multicellular organisms requires establishment of tissues with polarities in which cells have adopted strict orientation. The plant hormone auxin plays an essential role in this process controlling plant development and growth. Auxin signalling has been shown to occur through the ubiquitination and consecutive degradation via 26S proteasome of the Aux/IAA factors, which – in the absence of auxin – repress ARF proteins from regulating their targets. Different combinations of the 23 ARFs, the 29 Aux/IAAs and six TIR1/AFB auxin receptors in Arabidopsis have been proposed to contribute to the complexity of auxin responses during plant development.
Interestingly ETT/ARF3 (together with ARF13) is the only member of the ARF family which does not have the Aux/IAA interacting domains, and is therefore unlikely to function via the canonical auxin-signalling pathway. Mutations in the *ETTIN* (*ETT*) gene lead to dramatic gynoecium defects along the apical-basal axis with over-proliferation of apical tissue. However, ETT also functions throughout plant development, for example, during lateral root formation and in the establishment of leaf polarity and its expression is controlled at least partly by the transacting small interfering (*tasi*) RNA, *TAS3*. Despite the increasing knowledge about *ETT* function, a mechanistic model for how *ETT* may monitor auxin levels has not yet been proposed. In this project we hypothesise that it is through interaction with other proteins that ETT mediates its specific function within different tissues and developmental processes. In a first step towards testing this hypothesis, we performed a yeast-two-hydrid screen of the "REGIA" library of ~1,400 Arabidopsis transcription factors (TFs). This resulted in the identification of ETT-interactors in four different TF families. Detailed analyses of double and triple mutants as well as co-expression analyses are presented.

### P4-43 The effects of altered cytokinin levels on Arabidopsis leaf development

**Jan Skalák[1], Liesbeth Vercruyssen[2], Hannes Claeys[2], Stijn Dhondt [2], *Šárka Koukalová*[1], Dirk Inzé[2], Břetislav Brzobohatý[1]**
*[1]Laboratory of Plant Molecular Biology, Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i. and CEITEC-Central European Institute of Technology, Mendel University in Brno, Brno, Czech Republic;[2]Plant Systems Biology, VIB, Gent, Belgium*

E-mail of presenting author: skalak.jan7@gmail.com

Plants are multicellular organisms, which control their growth and morphogenesis by several biological processes to produce mature organs, such as leaves. At the cellular level, the leaf development consists of two main processes, cell proliferation and cell expansion. Differences in the timing, the rate and the spacing of these developmental processes determine the final size and structure of the leaves. Moreover, there is an interplay between the cellular processes and the differentiation of plastids into photosynthetically active chloroplasts which provide energy for the whole plant. Cytokinins (CKs) play a key role during many aspects of plant development, including chloroplast and leaf development. Constitutive reduction of CK content or signaling reportedly yields smaller leaves due to a decrease in cell proliferation, similar to the well-described function of CKs in maintaining cell division in the shoot apical meristem (SAM).Despite the extensive molecular knowledge on CKs action in the SAM, less information is available on how they affect cell division and the transition to cell expansion in leaves. Therefore, it is highly important to investigate the cellular changes during leaf growth induced by conditionally altered CK levels in parallel with the changes in gene expression. To this end, dexamethasone-inducible system was used to increase (*CaMV35S*>GR>*ipt*) or decrease (*CaMV35S*>GR>*HvCKX2*) CK content during specific stages of leaf 3 development in Arabidopsis seedlings. Both transgenic lines had smaller leaves after activation by dexamethasone. Cellular analysis revealed that increased levels of CKs prolong cell proliferation thereby diminishing cell expansion, while the lack of CKs suppresses cell division and enhances cell expansion to compensate for the reduced number of cells. Novel molecular events underlying the CK-dependent cellular responses in leaf 3 were revealed by RNA-seq based transcriptome profiling.

### P4-44 Disruption of cytokinin degradation and inactivation process during senescence doesn't prevent Arabidopsis thaliana from normal senescence progress

**Maria Smehilova[1], Jana Dobruskova[1], Ondrej Novak[2], Jan Humplik[2], Petr Galuszka[1]**
*[1]CRH – Department of molecular biology, Palacky University, Faculty of Science, Olomouc, Czech Republic; [2]CRH – Department of Chemical Biology and Genetics, Palacky University, Faculty of Science, Olomouc, Czech Republic*

E-mail of presenting author: maria.smehilova@upol.cz

Cytokinins are responsible for major physiological processes including senescence where nutrients are invested from source to sink tissues to ensure propagation of next generations. Cytokinin signalling of this process is crucial so in leaves an enormous decrease of cytokinin levels signalize to favour nutrient shift to sink organs. The decrease in *Arabidopsis thaliana* leaves is caused by cytokinin dehydrogenases CKX2 and CKX5. However, when a double knock-out of these two isomers was characterized no senescence delay was observed with only negligible redundancy effect of remaining CKX isoforms. Thus, cytokinin content was determined what showed increased levels of cytokinin glucosides. Therefore, cytokinin specific UDP-glycosyltransferases were analysed for their activity during senescence where the most upregulated isoform detected was *UGT85A1*. Consequently, the *UGT85A1* T-DNA insertional mutant was characterized and further used to prepare a triple knock out with *ckx2/ckx5*. The triple mutant *ckx2/ckx5/ugt85a1* was assessed for phenotype modifications, cytokinin content and expression profile of CK metabolism genes as well as for response to induced senescence, stress response or exogenous cytokinin treatment, respectively. Even though our results suggest that neither UGT85A1 nor CKX2 or CKX5 are the crucial key regulators in senescence process of *A. thalina* leaves our results show how *A. thaliana* modulates the cytokinin metabolism to maintain the proper homeostasis during senescence.
*Supported by Grant Agency of the Czech Republic no: P501/12/P160.*

### P4-45 Visualization of cell cycle progression using S/G2- and G2/M-specific markers

**Shiori Sugamata Aki[1], Hitomi Takagi[1], Ke Yin[1], Chikage Umeda-Hara[1], Minako Ueda[1], Masaaki Umeda[1]**
*[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), JST, CREST, Ikoma, Nara, Japan*

E-mail of presenting author: aki@bs.naist.jp

Real-time imaging of cell cycle progression is crucial to understand various developmental processes. In plants, the cell cycle marker is available only for the G2/M phase, while both S- and M-phase markers have been developed in human cells and zebrafish (termed 'Fucci'). The endocycle, which skips the M phase and repeats DNA replication, increases DNA content in individual cells, and the transition from the mitotic cell cycle to the endocycle has been shown to play a crucial role in organ development. To monitor not only the mitotic cell cycle but also the transition to the endocycle, it is essential to develop the S-phase-specific marker in plants.
We generated an RFP-fusion gene carrying a part of *Arabidopsis CDT1a*, which encodes a factor involved in DNA replication. Unexpectedly, this marker displayed the RFP fluorescence during the S-to-G2 phase, although *CDT1* has been reported to be expressed during the G1-to-S phase in human cells. We then combined this S/G2-specific marker with the G2/M-specific cyclin B1 marker, and established the cell cycle imaging system 'Cytrap (Cell-Cycle Tracking in Plant Cells)'. By using this system, we found that, just before entering the transition zone in roots, 4-5 cells showed the expression of only the S/G2 marker. This suggests that these cells are in the S/G2 phase of the last mitotic cell cycle, possessing the DNA content of 4C. We are also investigating the dynamic expression pattern of these markers under abiotic stress conditions.

### P4-46 Cytokinins promote rapid cell elongation in Arabidopsis roots

**Hirotomo Takatsuka[1], Masaaki Umeda[1]**
*[1]Department of biological sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, NARA, Japan*

E-mail of presenting author: h-takatsuka@bs.naist.jp

Sustainable root growth is accomplished by precise regulation of cell division and cell elongation. In *Arabidopsis* roots, cells produced in the proximal meristem are getting elongated through two-step transitions. During the 1st transition, cells enter the transition zone (TZ) and continue to grow gradually in the direction of both width and length. During the 2nd transition, cells enter the elongation/differentiation zone (EDZ). The mechanism underlying the 2nd transition is still largely unknown. Thus, to uncover the molecular mechanisms, we studied the effect of phytohormones on the 2nd transition. We found that cytokinin has a positive role in the 2nd transition in *Arabidopsis* roots. Our analyses also showed that dynamic actin rearrangement occurs along the 2nd transition. Indeed, *Arabidopsis* mutants with defects in actin organization showed a delay in the 2nd cell elongation. We are now investigating whether actin rearrangement is controlled downstream of cytokinin signaling which is involved in rapid cell elongation when entering the EDZ.

### P4-47 Auxin function during vegetative and reproductive organ development in Physcomitrella patens

**Mattias Thelander[1], Eric Pederson[1], Tom Viaene[2], Jiří Friml[3], Eva Sundberg[1], Katarina Landberg[1]**
*[1]Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Department of Plant Systems Biology, VIB, Gent, Belgium; [3]Institute of Science and Technology Austria, Klosterneuburg, Austria*

E-mail of presenting authors: Katarina.Landberg@slu.se, Mattias.Thelander@slu.se

In order to reveal the impact of local auxin biosynthesis and transport in the model moss *Physcomitrella patens* we have produced a number of genetic tools. We have previously shown that the two *P. patens SHI/STY* genes regulate auxin levels in moss, similar to *SHI/STY* genes in Arabidopsis that act as transcriptional activators of auxin biosynthesis genes. Also other seed plant gene families implicated in auxin function, including *YUCCA*- and *TAA1*-related biosynthesis genes as well as *PIN* and *Aux/LAX* transporter genes, are present in *P. patens*. We have established reporter, knockout and overexpressor lines for a selection of these genes to learn more about local auxin biosynthesis and transport in moss, focusing mainly on the protonemal vegetative tissue and reproductive organs.

## P4-48 Medicago truncatula WOX and PIN genes: participation in somatic embryogenesis

**Varvara Tvorogova[1], Maria Osipova[1], Ludmila Lutova[1]**
*[1]Genetics and biotechnology, Saint-Petersburg State University, Saint-Petersburg, Russian Federation*

E-mail of presenting author: krubaza@mail.ru

Somatic embryogenesis isn't widespread in nature, but there are a lot of in vitro systems for obtaining somatic embryos from many plant species. Most of them include cultivation of explants on media with auxin. In most cases, such treatment leads to callus formation and then, usually after auxin removal, embryos are formed on its surface. This process is easy to observe after embryos have reached globular stage, but it's much more difficult to study early stages of somatic embryo development, when embryos consist of one or several cells. Several genes of *WOX* family were shown to be involved in early stages of zygotic embryo development (1). Analysis of their expression during somatic embryogenesis can elucidate the first steps in this process.

A lot of studies suggest that formation of auxin gradient in the callus is the main cause of somatic embryogenesis; also it is shown that auxin gradient is important for zygotic embryos formation (2). Therefore it's very interesting to compare action of PIN auxin transporters in somatic and zygotic embryogenesis.

*Medicago truncatula* is a good model object for investigation of somatic embryogenesis. By using its embryogenic and non-embryogenic lines, we investigate to what extent somatic and zygotic embryogenesis are similar to each other. We found three *WOX* and three *PIN* genes of *M. truncatula*, which are supposedly involved in zygotic embryo formation, and measured their expression levels during somatic embryos formation. In embryogenic line, all analyzed *WOX* genes show an increase of expression level after cultivation on embryo-inducing media, but it isn't observed in non-embryogenic line. *PIN* genes which were analyzed didn't show a great difference in expression level between these lines. Now we perform the analysis of local expression of these *PIN* and *WOX* genes.

1)Breuninger et al., Dev. Cell, 2008
2)Pagnussat et al., Science, 2009

## P4-49 Meristematic characteristics of Agrobacterium-mediated tumors on pea

**Alena Vinogradova[1], Maria Osipova[1], Ludmila Lutova[1]**
*[1]Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russian Federation*

E-mail of presenting author: alena_08_93@mail.ru

Proliferation and differentiation of cells are controlled by plant hormones and transcription factors. Disorders of these processes lead to the development of various abnormalities, such as tumor formation. Crown gall tumors caused by *Agrobacterium tumefaciens* are widespread disease. The T-DNA from the bacterial tumor-inducing (Ti) plasmid, carrying genes involved in auxin and cytokinin biosynthesis, integrates into plants genome, leading to tumor formation. The role of transcription factors in tumor development is not well studied. It can be assumed that the same set of transcription factors that control cell proliferation normally can be involved in tumor formation. In our work we use *Agrobacterium*-mediated tumors induced on pea (*Pisum sativum*) hypocotyls as a model. First, we analyzed the distribution of proliferating cells in tumors using fluorescent-labeled thymidine analogue 5-Ethynyl-2´-deoxyuridine (EdU) which is incorporated into the DNA of proliferating cells. We found that proliferating cells in tumors are clustered into meristematic foci. To study if known meristem regulators are involved in *Agrobacterium*-mediated tumor development, we analyzed tumor development on pea mutants *Pssym28* and *Pssym29* defected in CLAVATA (CLV) components, CLV2 and CLV1-like kinase, respectively. No influence of pea *PsSYM29* and *PsSYM28* genes on tumor diameter was found, suggesting these genes do not control the development of *Agrobacterium*-mediated tumor in pea.

Next, we have analyzed the expression of several meristem-specific genes (*WOX*, *KNOX*, *CLE*, *PLT*, *SHR*, *SCR* families) upon the development of *Agrobacterium*-mediated tumors. Interestingly, we found that expression of *WOX5* gene was increased in all tumor samples, suggesting that key root meristem regulator WOX5 is involved in *Agrobacterium*-mediated tumor development. Together, our results will provide a better understanding of mechanisms linking abnormal balance of plant hormones leading to tumor formation and meristem-specific transcription factors.

This work was supported by RFBR 14-04-00591, 13-04-02140, 12-04-32021 grants, NIR SPbGU 1.38.676.2013, HIII-5345.2012.4.

### P4-50 Epidermis-derived cytokinin regulates the growth and development of shoot organs in Arabidopsis

**Sören Werner[1], Isabel Bartrina[1], Thomas Schmülling[1]**
*[1]Institute of Biology/Applied Genetics, FU Berlin, Berlin, Germany*

E-mail of presenting author: soeren.werner@fu-berlin.de

The phytohormone cytokinin plays a role in many aspects of plant growth and development. Although it is known for quite some time that a change of the cytokinin status in the plant can influence the size and structure of both aerial and subterranean organs, the regulatory mechanisms underlying organ size control by cytokinin are still unclear. In most instances the spatial organization of cytokinin action has not been resolved. We have generated transgenic *Arabidopsis* plants using L1- and epidermis-specific promoters to study the potential role of the outermost cell layer in regulating cytokinin-dependent developmental processes in shoot organs. Here we show that deregulation of the cytokinin content in the above-ground epidermis affects the size and the developmental status of leaves, thus influencing the juvenile to adult phase transition. The plants also display differences in height, suggesting a prominent role of epidermis-derived cytokinin in limiting shoot growth.

### P4-51 Molecular Mechanisms that Determine Pericycle Cell Identity

**Ye Zhang[1], Nobutaka Mitsuda[2], Chuan-Ming Yeh[2], Takeshi Yoshizumi[3], Yoichi Kondo[3], Masaru Takagi[2], Minami Matsui[3], Tatsuo Kakimoto[1]**
*[1]Department of Biology, Osaka University, Toyonaka, Japan; [2]National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan;[3]Plant Science Center, RIKEN Yokohama Institute, Yokohama, Japan*

E-mail of presenting author: zhangye@bio.sci.osaka-u.ac.jp
E-mail of corresponding author: kakimoto@bio.sci.osaka-u.ac.jp

Plant growth largely relies on a root system, which is developed post-embryonically through formation of lateral roots. In vascular plants, lateral roots arise from pericycle, a tissue layer surrounding the vasculature. In *Arabidopsis thaliana*, only pericycle cell-files contacting xylem (xylem pole pericycle, XPP), but not those contacting phloem are competent to produce lateral roots. Auxin induces formative cell division in the XPP. The molecular mechanisms underlying XPP specification are largely unknown. Our study aims to identify key transcriptional factors that regulate pericycle cell identity.

Specific expression of a transcription factor in one cell type may suggest its role in determining the cell identity. By analyzing cell-type specific microarray data, 70 transcription factor-coding genes that are preferentially expressed in XPP were selected as candidates. We overexpressed these genes alone and in fusion with a transcriptional activator VP16, or a transcription repressor SRDX in the XPP-specific GFP marker line J0121 plants. Then we screened for disrupted GFP expression patterns.

One candidate gene, No.17, appeared to be an important regulator of pericycle cell identity and lateral root development. Overexpression of No.17-SRDX causes loss of XPP-specific GFP expression in J0121 and inhibits lateral root formation. Activation of No.17's target genes by ubiquitous expression of No.17-VP16 caused ectopic expression of GFP in J0121. No.17-VP16 also induced ectopic expression of another pericycle marker *GATA23promoter*::NLS-GFP, suggesting that No.17 reinforces XPP cell identity. No.17 positively regulates *PIN1promoter*::PIN1-GFP expression, indicating No.17's possible role of modulating auxin polar transportation.

Overexpression of another candidate gene, No.51, could cause ectopic expression of XPP-specific GFP in J0121. Auxin treatment induced cell division of GFP-expressing cells in root epidermis. Lateral root formation was also affected in the No.51-overexpressing plants. Ongoing studies on gene No.17, No.51 and their possible interactions may serve to reveal the regulatory network that determines XPP cell identity.

# SESSION 5: HORMONE CROSS-TALK

## O5-1 A secret crosstalk of peptide, auxin and brassinosteroid signalling in lateral root development

**Ildoo Hwang**[1]
*[1]Department of Life Sciences, POSTECH Biotech Center, Pohang University of Science and Technology, Pohang, South Korea*

E-mail of presenting author: ihwang@postech.ac.kr

The phytohormone auxin is a key developmental signal in plants. Perception of auxin maxima determines the signalling output eventually by releasing ARFs from AUX/IAA repressors. However, how this signalling circuit determines auxin sensitivity for diverse developmental processes remains elusive. Recently, we showed that phosphorylation of ARF7 and ARF19 via TDIF–TDR-mediated activation of BIN2 potentiates auxin signalling output during lateral root organogenesis. BIN2 directly interacted with ARF7 and ARF19, and induced their phosphorylation *in planta*. BIN2-mediated phosphorylation of ARF7 and ARF19 suppressed their interaction with AUX/IAAs, and subsequently enhanced the transcriptional activity to their target genes *LBD16* and *LBD29*. TDIF-initiated TDR signalling directly acts on BIN2-mediated ARF phosphorylation, leading to the regulation of auxin signalling during lateral root development. In summary, our study reveals a new signalling cascade in lateral root organogenesis involving the auxin perception-independent regulation of interaction between ARF and AUX/IAA proteins via the TDIF–TDR–BIN2 module.

## O5-2 Hormonal regulation of root system architecture

**E. Benkova**[1,2], **C. Cuesta**[1], **M. Simaskova**[3]
*[1]Institute of Science and Technology Austria Austria, Am Campus 1, 3400 Klosterneuburg, Austria; [2]Mendel Centre for Plant Genomics and Proteomics, Masaryk University, CEITEC MU, CZ-625 00 Brno, Czech Republic; [3]Department of Plant Systems Biology, VIB and Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, B-9052 Gent, Belgium*

E-mail of presenting author: eva.benkova@ist.ac.at

The plant hormones auxin and cytokinin are central endogenous signaling molecules that regulate root growth lateral root organogenesis. Stimulatory effect of auxin is counterbalanced by cytokinin, and thus, tight control and mutual balance of their antagonistic activities are particularly important during the early phases of lateral root organogenesis to ensure continuous lateral root initiation and proper development of lateral root primordia. Our studies revealed how cytokinin contributes to spatiotemporal regulation of lateral root organogenesis. We analyzed cytokinin and cytokinin signaling distribution along the root and showed that the early phases of lateral root organogenesis including priming and initiation take place in the root zone with strongly repressed cytokinin responses. Further progress in our understanding of auxin-cytokinin crosstalk in regulation of root system architecture using genomic approach will be discussed.

## O5-3 Apical dominance

**Christine Beveridge[1], Michael Mason[1], John Ross[2], Benjamin Babst[3], Brittany Weinclaw[3], Stephanie Kerr[1], Renyi Gui[4]**
*[1]Biological Sciences, The University of Queensland, Brisbane, Australia; [2]Plant Science, University of Tasmania, Hobart, Australia; [3]Biosciences Department, Brookhaven National Laboratory, New York, USA; [4]Bamboo Research Institute, Zhejiang Agriculture and Forestry University, Zhejiang, China*

E-mail of presenting author: c.beveridge@uq.edu.au

Growing shoot tips can maintain dominance over the growth of axillary buds in a process termed apical dominance. This is typically thought to be under the control of the basipetal flow of auxin from the shoot tip. Here we show that the shoot tip, which is also a hungry consumer of mobile sugars, acts to prevent the initial growth of axillary buds by affecting the levels of sugars available for the buds. Mobile photoassimilates/ sugars move at 150 cm per hour in tall garden pea plants. After decapitation, photoassimilate supply to the lower stem is enhanced at a rate of about 0.5 cm per minute and correlates with the timing of bud outgrowth. Exogenous sucrose can induce bud release in intact plants in a dynamic response similar to decapitated plants. Sucrose induces the same transcription factor in buds previously reported to be regulated by plant hormones to control bud outgrowth (*BRC1, BRANCHED1*). In contrast, auxin moves at about 1 cm per hour and too slowly for auxin depletion after decapitation to induce the early growth of axillary buds. Treatments that reduce auxin content do not lead to bud release unless sugars are also increased by these treatments. Sucrose is therefore both necessary and sufficient to induce bud release. Auxin treatment to the cut surface of decapitated shoots does not prevent the early growth of buds and an auxin transport inhibitor supplied directly to axillary buds of decapitated plants also fails to prevent early bud outgrowth. This discovery establishes a paradigm for apical dominance, whereby sugar supply, controlled by shoot-tip demand, has an important regulatory role in the initial release of buds for growth and that the plant hormones, auxin, strigolactone and cytokinin gain in importance once this release has occurred. These findings published in PNAS will be updated with recent work on auxins, cytokinins and strigolactones

## O5-4 ABA and cytokinin regulation of ABCB4 abundance at the plasma membrane

**Angus Murphy[1], Doron Shkolnik[1]**
*[1]Dept. of Plant Science and Landscape Architecture, University of Maryland, College Park, USA*

E-mail of presenting author: asmurphy@umd.edu

ABCB/PGP auxin transporters are regulated by phosphorylation and FKBP42/TWD1. Arabidopsis ABCB4 regulates auxin homeostasis in the root epidermis and is a target of the herbicidal auxin 2,4-D. *ABCB4* expression is positively regulated by auxin in root hair cells, but negatively regulated in atrichoblasts. *abcb4* phenotypes are sensitive to sucrose and osmotic stress. *ABCB4* expression is positively regulated by abscisic acid (ABA) and cytokinin, but not ethylene. The *ABCB4* promoter contains two full cis-acting elements bound by ABI4, which positively and negatively regulates multiple ABA response proteins. Quantitative real time PCR analysis showed that *ABCB4* transcript levels are 2-3 time higher in abi4mutants. Surprisingly, $ABCB4_{Pro}$ ABCB4-GFP abundance was reduced after treatment with ABA and cytokinin in the wild type, but not in the abi4 background, suggesting an ABI4-regulated post-translational regulatory mechanism. ABI4 was subsequently found to directly regulate Aspartatyl Protease A2 (APA2). Loss of *ABCB4* results in reduced lateral root formation and shootward transport from the root apex, while *apa2* and *abi4* mutants exhibit enhanced lateral root formation and increased shootward IAA transport. $ABCB4_{Pro}$ ABCB4-GFP was found to be significantly more stable in *abi4* and *apa2* following ABA, cytokinin and auxin treatment. Recombinant APA2 specifically cleaves ABCB4 *in vivo* and *in vitro*. APA2 localizes to the plasma membrane and *trans* Golgi network, suggesting that sphingolipid and sterol – enriched membranes are sites of ABCB4 cleavage by APA2. These results indicate the presence of an unanticipated mechanism that functions in ABA and cytokinin-dependent regulation of plasma membrane proteins.

## O5-5 The role of cytokinin and ethylene signaling involvement in the multistep phosphorelay pathway

**Markéta Žďárská[1], Vendula Hrdinová[1], Bedřich Pešek[2], Jiří Malbeck[2], Jan Hejátko[1]**
*[1]Research group Functional Genomics and Proteomics of Plants, Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [2]Laboratory of Mass Spectrometry, Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Prague, Czech Republic*

E-mail of presenting author: zdarska@sci.muni.cz
E-mail of corresponding author: hejatko@sci.muni.cz

Cytokinins (CKs) and ethylene are known as principal regulators of several physiological and developmental processes in Arabidopsis. Both phytohormones are recognized by proteins from the same sensor histidine kinase family. However, CK signal is mediated via multistep phosphorelay (MSP), while ethylene signaling occurs through MAP-kinase (MAPK) cascade. Both signaling pathways are suspected to interact for a long time but the experimental evidence is still limited.
Here we show that CK and ethylene signaling are interconnected via action of CK receptors AHK2 and AHK3 and ethylene receptor ETR1. We demonstrate that both hormones, CK and 1-aminocyclopropane-1-carboxylic acid (ACC), the rate-limiting precursor of ethylene biosynthesis, are able to induce the type-A *ARABIDOPSIS RESPONSE REGULATORS* (*ARRs-A*) reflecting the activity of the MSP-mediated CK signaling pathway. Using mutants with impaired CK perception we demonstrate that CK signaling interferes with the ethylene-mediated activation of MSP with dominant contribution of AHK2 and AHK3. Moreover, we show that CK receptors regulate the endogenous ACC levels. On the other hand, CK mediates differential expression of *ARRs-A* in the WT and *etr1-1* mutant, suggesting the role of ETR1 in the CK-dependent upregulation of MSP. We also observed complete resistance in the CK-induced root shortening and CK-mediated reduction of the root apical meristem (RAM) size in the *etr1-1* line suggesting the so far unknown role of ethylene in the CK-dependent regulation of RAM size. Finally, we demonstrate the ability of ETR1 to interact with a subset of ARABIDOPSIS HISTIDINE-CONTAINING PHOPHOTRANSFER PROTEINS (AHPs), the signaling intermediates acting downstream of CK receptors.
Overall, our findings propose the integration of both CK and ethylene signaling in single MSP pathway and an intense mutual CK-ethylene crosstalk in MSP-mediated signaling.
*Supported by CZ.1.07/2.3.00/30.0009 and 13-25280S.*

## O5-6 Ethylene – cytokinin interplay in regulation of PIN1 trafficking

**Peter Marhavy[1], Anas Abuzeineh[2], Ellie Himschoot[2], Steffen Vanneste[2], Jiří Friml[1], Eva Benková[1]**
*[1]IST Austria, IST Austria, Klosterneuburg, Austria; [2]Department of Plant Systems Biology, Ghent University, VIB, Gent, Belgium*

E-mail of presenting author: pmarhavy@ist.ac.at

Plant hormones including auxin, cytokinin and ethylene play an important role in the regulation of plant growth and development. A complex network of interactions and feedback circuits interconnects these hormonal pathways and determines the final outcome of the individual hormone actions. Well-established are the mutual regulations of metabolic and signaling pathways, as well as the modulation of auxin transport by cytokinin and ethylene. Cytokinin was shown to influence cell-to-cell auxin transport by modification of the expression of several auxin transport components and thus to modulate auxin distribution important for root development. Recently, we identified alternative mode of cytokinin action that uses endocytic trafficking as a means to direct plant organogenesis. We showed that cytokinin regulates recycling of the auxin efflux carrier PIN1 to the plasma membrane by redirecting it for lytic degradation in vacuoles. This rapid, non-transcriptional regulation of the PIN1 abundance enables a precise control of auxin fluxes and distribution during LR organogenesis and other cytokinin-mediated developmental regulations, such as root meristem differentiation. Our recent results indicate that ethylene might contribute to regulation of PIN subcellular trafficking and proper positioning of PIN1 transporters at membranes.

## O5-7 Strigolactone is required for systemic cytokinin homeostatic feedback loops

**Colin Turnbull[1], Ioanna Antoniadi[1], Nicolas Kral[1], Neelam Chaudhary[1], James Strutt[1], Mark Bennett[1]**
*[1]Department of Life Sciences, Imperial College London, London, UK*

E-mail of presenting author: c.turnbull@imperial.ac.uk

Cytokinins move readily between shoot and root through phloem and xylem, and may act as systemic growth and stress signals. However, we do not fully understand the regulation of cytokinin pool sizes and cytokinin transport. Isopentenyl transferase (*IPT*) genes encode cytokinin biosynthetic enzymes, with further conversion to (trans)-zeatin cytokinins catalysed by CYP735A enzymes. Based on reporter gene profiles, spatial expression pattern of each family member is tightly regulated, yet single gene knockouts have little effect on phenotype or overall cytokinin pool sizes, suggesting systemic complementation due to cytokinin mobility. Cytokinin transport is dominated by isopentenyl adenine (IP) cytokinins in phloem sap, whereas zeatin types are most abundant in xylem. Here we tested three hypotheses: first, that compensatory cytokinin movement should occur in grafts between shoots and roots carrying multiple knockout mutations for either *IPT* or *CYP735A* genes ($CK^-$ genotypes); second, that recirculation of shoot-biosynthesised cytokinins should be detectable in xylem sap of certain graft combinations; and third, that strigolactone ($SL^-$) mutations which cause greatly reduced xylem cytokinin content may impact on the normal cytokinin homeostasis loops, resulting in attenuated hormonal and phenotypic complementation. Wild-type Arabidopsis shoots retained normal phenotypes on all grafted rootstock genotypes including $CK^-$ $SL^-$ doubly deficient lines (*max4 ipt3,5,7* or *max4 cyp735a1,a2)*. Likewise wild-type rootstocks were able to rescue shoot phenotypes of all $CK^-$ and $SL^-$ biosynthetic mutants. Reciprocal wild-type grafts to $CK^-$ mutants had elevated xylem cytokinin content, indicative of compensatory systemic cytokinin transport. Extreme dwarf and branching $CK^-$ $SL^-$ shoots were rescued in shoot size if grafted to $SL^-$ roots. In contrast, $SL^-$ shoots of the reciprocal graft to $CK^-$ $SL^-$ roots unexpectedly displayed a dwarf phenotype. Both these results suggest a role for root-sourced cytokinins in regulating shoot growth, and we discuss how strigolactone defects might perturb the normal systemic cytokinin homeostasis mechanisms.

## O5-8 Strigolactones reduce lateral rooting via altered cytokinin responses and differential auxin transport in the shoot-root junction

**Stephen Depuydt[1], Lingxiang Jiang[1], Cedrick Matthijs[1], Belen Garcia-Marquez[1], Sofie Goormachtig[1]**
*[1]WE09/Plant Systems Biology, UGent/VIB, Gent, Belgium*

E-mail of presenting author: stpuy@psb.ugent.be

Strigolactones have been well known as host detection cues for root parasitic plants and for symbiotic interactions with arbuscular mycorrhiza. Due to their recently uncovered roles in shoot branching inhibition, they are now fully recognized as plant hormones with multiple functions. It has been demonstrated that strigolactones endogenously influence the root system architecture as well, but insights in the molecular mechanisms are still lacking. Here, we addressed the cross-talk between strigolactones, cytokinins and auxins during lateral root development. We show that lateral root development is transiently affected by strigolactone treatment, causing a problem mainly in the emergence of the distal lateral roots, that show altered cytokinin signaling. Our data clearly imply the specific cytokinin signaling components AHK3 and ARR1/ARR12 and the SHY2/IAA3 module in mediating the SL response to reduce lateral root formation since loss of function mutants are non-responsive towards the synthetic strigolactone GR24. Furthermore, our data point towards differential PIN expression in the vasculature of the shoot-root junction as the underlying cause of the reduction in lateral root development upon strigolactone treatment. By increasing the auxin content, either via overexpression of PIN1 or YUCCA, or by exogenously applying PIN transportable auxins, the effect of strigolactones on lateral rooting can be reverted, while blocking auxin transport from the shoot will render strigolactone-insensitive plants completely sensitive towards strigolactones. Altoghether our data show that strigolactone effects on lateral rooting are dependent of the hormonal landscape in the root, resulting from intimate cross-talk between auxins and cytokinins, the two main players in lateral root formation.

### O5-9 Apical dominance is controlled by interaction between cytokinin and auxin biosynthesis/degradation in stem and axillary buds

**Hitoshi Mori[1], Mina Tanaka[2], Sae Sato-Shimizu[1], Rika Sugiura[1], Hitoshi Sakakibara[3]**
*[1]Bioagricultural Sciences, Nagoya university, Nagoya, Japan, [2]Home Economics, Gifu Women's University, Gifu, Japan; [3]Center for Sustainable Resource Science, RIKEN,*

E-mail of presenting author: morihito@agr.nagoya-u.ac.jp

Apical dominance is a phenomenon in which a terminal bud inhibits the outgrowth of axillary buds. Although involvement of auxin, which represses axillary bud outgrowth, and cytokinin (CK), which promotes axillary bud outgrowth, has been proposed, little is known about the underlying molecular mechanisms. Firstly, we demonstrated that auxin negatively regulates local CK synthesis in the nodal stem by controlling the expression level of the gene pea *adenosine phosphate–isopentenyltransferase (PsIPT)*, which encodes a key enzyme in CK biosynthesis. Before decapitation, *PsIPT1* and *PsIPT2* transcripts were undetectable; after decapitation, they were markedly induced in the nodal stem along with CK accumulation. *PsIPT* expression was repressed by the application of indole-3-acetic acid (IAA). In excised nodal stem, *PsIPT* expression and CK levels also increased under IAA-free conditions. On the other hand, in a new shoot apex, which had previously been a dormant axillary bud, YUCCA and PsPIN1 were expressed 3 h and 6h after decapitation respectively. *de novo*-synthesized IAA derived not only flowed to the stem 9 h after decapitation and again repressed *PsIPT* expression, but also induced gene expression of CK oxidase. As the result, CK levels in the stem were low again. These results indicate that, in apical dominance, one role of auxin is to control local CK level in the nodal stem.

### O5-10 PIN proteins: "A playground" for strigolactone-auxin cross-talk in roots

**Hinanit Koltai[1]**
*[1]Institute of Plant Science, ARO, Volcani Center, Bet Dagan, Israel*

E-mail of presenting author: hkoltai@agri.gov.il

Strigolactone, the new plant hormone, is being perceived through a cellular signaling module of D14/MAX2/SCF that by ubiquitination targets repressors for degradation. Recently, we found evidence suggesting a non-cell autonomous mode of action for strigolactones, that might underlie the cross-talk of strigolactones with auxin. One is the spatial requirements for MAX2 dependent strigolactone signaling for the execution of some of their activity, suggesting that strigolactone signaling may act at a short-range, across cells and cell layers. Further evidence suggests that strigolactones are associated with changes in PIN protein localization in the plasma membrane within the root epidermis. Furthermore, we found that strigolactones modify, in a MAX2-dependent fashion, cell trafficking and cytoskeleton, in association with their effect on PIN2 polar localization and PIN2 endocytosis. Together, these findings suggest, firstly, that although strigolactones are being perceived in a cell autonomous way, at least some aspects of their activity are carried in a non-cell autonomous fashion. Secondly, these findings of strigolactone effect on PIN trafficking and plasma membrane localization better explain the cross talk between strigolactones and auxin in terms of regulation of auxin flux in the root. It is also possible that the regulation of PIN activity is a "playground" for network interaction between different hormones, including auxin, ethylene, cytokinin and now, strigolactones, for regulation of root development.

## O5-11 Root growth is modulated by differential hormonal sensitivity in neighboring cells

**Yulia Fridman[1], Sigal Savaldi-Goldstein[1], Liron Elkouby[1], Neta Holland[1], Kristina Vragović[1], Rivka Elbaum[2]**
*[1]Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel, [2]Smith Institute of Plant Sciences and Genetics in Agriculture, Hebrew University of Jerusalem, Rehovot, Israel*

E-mail of presenting author: sigal@technion.ac.il

Coherent plant growth requires spatial integration of hormonal pathways and cell wall remodeling activities. However, the mechanisms governing sensitivity to hormones and how cell wall structure integrates with hormonal effects are poorly understood. We find that coordination between two types of epidermal root cells, hair- and non-hair cells, establishes root sensitivity to the plant hormones brassinosteroids. While expression of the brassinosteroid receptor BRI1 in hair cells promotes cell elongation in all tissues, its high relative expression in non-hair cells is inhibitory. Elevated ethylene and auxin, and deposition of crystalline cellulose underlie the inhibitory effect of BRI1. We propose that the relative spatial distribution of BRI1, and not its absolute level, fine-tunes growth.

## O5-12 Elucidating cytokinin two-component signaling crosstalk to plant immune networks: A systems biology perspective

**Muhammad Naseem[1], Thomas Dandekar[1], Meik Kunz[1]**
*[1]Department of Bioinformatics, University of Wuerzburg, Wuerzburg, Germany*

E-mail of presenting author: dandekar@biozentrum.uni-wuerzburg.de
muhammad@biozentrum.uni-wuerzburg.de

Molecular genetic studies have helped to elucidate a multistep two-component cytokinin (CK) signaling system[1] in *Arabidopsis*.The importance of CK signaling in controlling plant growth and development has thoroughly been investigated[1,2]. The efforts highlighting its implications in plant immunity are lagging behind. Higher plant CK levels and signaling correlate with increased plant resistance against various pathogens[3,4,5]. However, the underlying molecular mechanisms whereby CK signaling modulates plant immune networks are not fully understood. CK contents and signaling increase plant resistance[6,7], pathogens may modulate CK signaling in the host plant in the opposite way.
To analyze these changes in CK signaling upon pathogen infection we adapted systems biology approaches. We mapped high quality Arabidopsis Protein-Protein-Interactions (Y2H)[8] and investigated their changes in gene expression after treatment with *Pst* DC3000. We integrated identified nodes of the cellular interactome enriched in immune functions and their interacting partners into sub-networks as per our published methodology. According to our analysis, even seventeen out of 27 nodes relevant to CK canonical signaling pathways are involved in our immune sub-network. Based on different criteria, we identified functional hubs in our sub-networks. Two of the CK signaling proteins AHK2 (receptor) and ARR14 (type-B-regulator) are among the top ten most connected hubs. Besides, we also conducted analyses on modulated CKs conditions such as deletion of type-A ARRs as well as exogenous CK application. These analyses culminate in a functional care module where nodes of SA mediated defense and cytokinins pathways actively participate. Furthermore, based on our established Boolean model[4,5] (plant-immune and pathogen-virulence-specific-network) we conducted dynamic *in-silico* simulations of the plant immune response.
In summary, our proposed talk will present our own developed combined systems biology methodology. We identify immune relevant functional hubs and show an integrated analysis of the *Pst* DC3000 and *Arabidopsis* interaction with focus on the hormonal aspects of plant immunity.

## O5-13 N6-adenosyl methylation regulates vascular development and hormonal response in Arabidopsis

**Kamil Ruzicka[1], Sedeer El-Showk[2], Ana Campilho[2], Vasiliki Zacharaki[3], Muhammad Kashif[2], Jan Hejatko[1], Geert De Jaeger[4], Gordon Simpson[3], Rupert Fray[5], Ykä Helariutta[2]**

*[1]Dept. Funct. Genomics Proteomics, CEITEC Masaryk University, Brno, Czech Republic), [2]Wood Development Group, Institute of Biotechnology, Helsinki, Finland, [3]Plant Sciences Division, Dundee University, Dundee, UK, [4]Department of Plant Biotechnology and Genetics, Department of Plant Systems Biology, VIB, Gent, Belgium, [5]Plant Sciences Division, University of Nottingham, Nottingham, UK*

E-mail of presenting author: kamil.ruzicka@ceitec.muni.cz
E-mail of corresponding author: yrjo.helariutta@helsinki.fi

$N^6$-adenosine methylation ($m^6A$) is the most common mRNA modification in eukaryotes. However, the physiological role of $m^6A$ and the underlying molecular machinery remain practically unknown. It is very little known about its role in pattern formation – in plants, as well as in other eukaryotes.

Protein AHP6, a member of cytokinin signaling cascade, plays a critical role during protoxylem development and is the earliest known marker of protoxylem identity. Expression of AHP6 is also directly regulated by auxin. We isolated mutant *emb2016-6*, which shows a reduced *AHP6prom:GFP* activity, accompanied with aberrant protoxylem formation. *emb2016-6* shows auxin related defects and resistance to auxinic compounds 2,4-D and NPA, and also to ethylene.

*emb2016-6* codes for a weak allele of an embryonically lethal gene. It is, based on its similarity to *Drosophila melanogaster* homologs, presumably involved in mRNA processing. In order to elucidate the molecular function of EMB2016 complex further, we performed two rounds of independent protein interaction hunts. We co-purified $m^6A$ methylase and a protein homologous to EMB2016 interactors from Drosophila. We demonstrate that knockdown lines of proteins closely associating with EMB2016 show depleted *AHP6prom:GFP* expression, vascular defects and altered sensitivities to 2,4-D. We reveal that *emb2016-6* has dramatically reduced levels of $m^6A$ in mRNA and EMB2016 thus appears to be a novel factor required for $m^6A$ in plants and also in other eukaryotes.

*Supported by Czech Science Foundation (P501/12/0934) and by the European Social Fund (CZ.1.07/2.3.00/20.0189 and CEITEC).*

## O5-14 The interaction between glucose and cytokinin signal transduction pathway in *Arabidopsis thaliana*

**Sunita Kushwah[1], Ashverya Laxmi[1]**

*[1]NIPGR, National Institute for Plant Genome Research, Delhi, India*

E-mail of presenting author: kushwah.sunita@gmail.com
E-mail of corresponding author: ashverya_laxmi@nipgr.ac.in

Sugar and Cytokinins (CKs) are fundamental to plants and regulate a number of similar processes. In this study, whole genome transcript profiling along with physiological analysis has been performed to find out the interdependence/overlap between glucose (GLC) and CK signaling in Arabidopsis seedlings. GLC could transcriptionally affect 76% of CK-regulated genes at whole genome level, 89% of which were agonistically regulated. We have found that in addition to coiling and waving, aspects of optimal root architecture GLC and CK together also control a novel root directional response that we termed as "CK-induced root growth response".Arabidopsis vertically grown seedling root transiently deviate away from CK containing medium and revert back to it showing a hopping pattern of root growth. Asymmetrical exposure of CK at the root tip promotes cell elongation that is increased by GLC in hexokinase-influenced, G protein independent manner. The response is reduced in CK receptor AHK4 and type-B ARR mutants, while type-A ARR mutants show enhanced response. Ethylene resistant mutants, ETR1 and EIN2 exhibit reduced response suggesting that CK works through ethylene to exhibit this response. Auxin transport facilitated by PIN2 as well as auxin signaling through control of the steady-state level of transcriptional repressors IAA7, IAA14, and IAA17 via TIR1/AFB are also involved in CK-induced root growth response. Actin filament organization is also important for this response. GLC and CK could also regulate root length, root gravitropism, lateral roots and root hair initiation and elongation. CK signaling mutant analysis suggested that CK-receptor, type B and type A ARRs are involved in various GLC induced root growth and developmental changes. Both GLC and CK signaling can not alter root length in light in auxin signaling mutant *IAA17* suggesting that they may involve auxin signaling component as a nodal point.

# POSTERS

## P5-1 The outgrowing orchestra of pea axillary buds is conducted by auxin canalization under cytokinin and strigolactone fine-tuning

**Jozef Balla[1], Zuzana Medveďová[2], Petr Kalousek[3], Nela Daňková[2], Vilém Reinöhl[2], Stanislav Procházka[2]**
*[1]CEITEC MENDELU, Department of Plant Biology, Mendel University, Brno, Czech Republic; [2]CEITEC MENDELU, Mendel University, Brno, Czech Republic; [3]Department of Plant Biology, Mendel University, Brno, Czech Republic*

E-mail of presenting author: xballa@mendelu.cz

The regulatory mechanism, by which the shoot apical meristem (SAM) controls outgrowth of axillary meristems, is known as apical dominance. Removal of the SAM releases one or more axillary buds from their dormancy to replace the previously dominant apex. The most studied signal molecule originated in the SAM is auxin. Its polar transport mediated by PIN auxin transporters in the stem is necessary for the control of bud outgrowth by a dominant SAM. After decapitation the axillary buds establish directional auxin export by subcellular polarization of PIN proteins, while auxin application on the decapitated stem prevents this PIN polarization and canalization of laterally applied auxin. Direct application of cytokinins to axillary buds can promote their outgrowth, even in intact plants, while direct application of strigolactone can inhibit their outgrowth.
On a two-nodal-bud pea model system by auxin transport inhibitor (TIBA), endocytosis inhibitor (BFA) or proteosynthesis inhibitor (cycloheximide) as well as cytokinin and strigolactone treatment we demonstrated the central role of auxin and its export into the main stem for regulation of bud outgrowth competition and also for long-range signalling for bud outgrowth. Further, we showed that cytokinins and strigolactone influence auxin transport network properties by modulation of PIN auxin transporters. The effect of the applied inhibitors and plant growth regulators was characterized by morphological and molecular responses of the buds (e.g. bud growth, expression profiles of dormancy marker gene, auxin influx and efflux transporters, cell cycle related gene etc. and PIN1 immunoanalysis).
*This work was supported by the project „CEITEC – Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068).*

## P5-2 Sucrose promotes cytokinins synthesis and auxin export during axillary bud outgrowth

**François Barbier[1,2], Thomas Péron[1,2], Marion Lecerf[1,2], Maria-Dolores Perez-Garcia[1], Quentin Barrière[1], Jakub Rolčík[3], Stéphanie Boutet-Mercey[4], Sylvie Citerne[4], José Le Gourrierec[5], Jessica Bertheloot[2] and Soulaiman Sakr[1]**
*[1]Agrocampus-Ouest, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 149 QUASAV, F-49045 Angers, France; [2]INRA, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 149 QUASAV, F-49071 Beaucouzé, France; [3]Laboratory of Growth Regulators, Faculty of Science, Palacký University and Institute of Experimental Botany AS CR, Šlechtitelů 11, 78371 Olomouc, Czech Republic; [4]Institut Jean-Pierre Bourgin, Unité Mixte de Recherche 1318, Institut National de la Recherche Agronomique–AgroParisTech, Institut National de la Recherche Agronomique Centre de Versailles-Grignon, 78026 Versailles cedex, France; [5]Université d'Angers, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 149 QUASAV, F-49045 Angers, France*

E-mail of presenting author: francois.barbier@agrocampus-ouest.fr

Bud outgrowth is under the control of a complex hormonal network involving auxin, cytokinins (CK) and strigolactones (SL). Apical-derived auxin flowing in the main stem inhibits buds outgrowth on the same axis in a mechanism called apical dominance. In the one hand, auxin inhibits the synthesis of CK, a positive regulator of bud outgrowth, and induces the synthesis of SL, a class of inhibitor. On the other hand, auxin prevents axillary buds to export their auxin into the main stem, which is required to allow them to grow out. More recently, nutrients, and more precisely sugars, have been shown to promote early bud outgrowh, even in the presence of auxin in the main stem. However, how sugars impact on the regulatory network controlling bud outgrowth remains largely unknown. Here, we show that sucrose supply to *in vitro* cultivated buds (i) induces CK synthesis and inhibits SL signalling in the stem and (ii) induces auxin synthesis and export from buds, allowing thus buds to grow out. Morever, during this process, sucrose is involved in a signalling pathway since its non-metalizable analogs were also able to trigger bud outgrowth in different species. These current trends make sucrose a key endogenous regulator, actively participating in the induction of bud outgrowth by interacting with the hormonal network controlling this process.

## P5-3 A forward genetic screen reveals PAC7 as auxin-cytokinin crosstalk component involved in lateral root organogenesis

**Lucie Crhak Khaitova[1], Anas Abuzeineh[2], Agniezska Bielach[2], Peter Marhavy[3], Eva Benkova[3]**
*[1]Department of Functional Genomics and Proteomics, Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic; [2]Department of Plant System Biology (VIB), Department of Plant Biotechnology and Bioinformatics (Ghent University), VIB and Ghent University, Gent, Belgium; [3]Institute of Science and Technology Austria, Klosterneuburg, Austria*

E-mail of presenting author: lucie.khaitova@ceitec.muni.cz

Auxins and Cytokinins are two major groups of phytohormones that play role in different plant growth and developmental processes, ranging from cell division and cell differentiation to organogenesis. The interaction and relationship between those two hormones among root development has been recently studied, showing that they interact antagonistically. The molecular mechanism behind these interactions is yet to be elucidated. During lateral root (LR) development, auxin positively regulates the LR initiation and development. On the other hand, cytokinin negatively regulates LR development by inhibiting LR formation. A forward genetic study has been performed to identify molecular components that mediate the auxin-cytokinin crosstalk and are involved in LR formation. Screening of mutants that produce LRs after applying auxin simultaneously with inhibiting concentrations of cytokinin was performed. 22 mutants denoted as **p**rimordia on **a**uxin and **c**ytokinin (pac) were selected and classified based on their LR initiation and response to auxin and cytokinin. Part of the mutants showed high LR formation, indicating that they are possibly involved in the regulation of LR initiation, whereas others, which were cytokinin resistant, represent known as well as novel components of the cytokinin signaling.Further characterization of these mutants would shed light on the regulatory pathways of the auxin-cytokinin crosstalk. *pac7*, which was found to be highly resistant to cytokinin and moderately resistant to auxin, is being further studied. In dark conditions, it showed strong defects in its development, e.g. short hypocotyl, open cotyledons and defects in the apical hook. Progress in the analysis of the *pac7* mutant as well as the predicted gene function will be discussed.

## P5-4 Tissue specific cross-talk between cytokinins and auxin is critical for Arabidopsis root meristem growth and development

**Riccardo Di Mambro[1], Micol De Ruvo[2], Serena Perilli[3], Rosangela Sozzani[4], Elena Pacifici[1], Benfey Philip[5], Wolfgang Busch[6], Costantino Paolo[1], Sabrina Sabatini[1]**
*[1]Biology and Biotechnology „C. Darwin", „Sapienza" University of Rome, Rome, Italy; [2]Faculty of Engineering, University CAMPUS BioMedico, Rome, Italy; [3]Department of Plant Developmental Biology, Wageningen University Research, Wageningen, The Netherlands; [4]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, USA; [5] Department of Biology and Duke Center for Systems Biology, Duke University, Durham, USA; [6] Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria*

E-mail of presenting author: riccardo.dimambro@uniroma1.it

The establishment of a dynamic equilibrium between cell division and cell differentiation guarantee *Arabidopsis* root meristem growth and mainteance. This equilibrium depends on the antagonistic interaction of two plant hormones cytokinin and auxin. We have previously shown that from the vascular tissue of the root meristem transition zone, cytokinins prompt cell differentiation, while auxin promotes cell division of all the other root tissues. We now have evidence that cytokinins control root meristem size from another root tissue type, the lateral root cap (LRC). We have observed that plants with low level of cytokinins in the LRC have an enlarged meristem and enhanced root growth. In contrast, when cytokinin signaling is enhanced in the LRC, plants display short meristems and short roots. Genetic and molecular analysis and root modelling simulations suggest a novel mechanism in which cytokinins, in order to fine tune auxin availability, regulate the level of biological active forms of auxin in specific root tissues thus controlling auxin gradient distribution.

### P5-5 Light-regulated expression of sensor histidine kinase CKI1 controls cytokinin-related development of Arabidopsis

**Tereza Dobisová[1], Vendula Hrdinová[1], Candela Cuesta[2], Romana Hejátková[1], Eva Benková[2], Jan Hejátko[1]**
*[1]Laboratory of Molecular Plant Physiology and Functional Genomics and Proteomics of Plants, CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [2]Institute of Science and Technology Austria, Vienna, Austria*

E-mail of presenting author: t.kolouchova@seznam.cz

In plants, the multistep phosphorelay (MSP) pathway mediates a range of regulatory processes, including those activated by the plant hormones cytokinins. Cytokinin sensitivity depends on light; however, the molecular mechanisms underlying the interaction between light and cytokinin signaling remain elusive. In the screen for upstream regulators, we identified a *LONG PALE HYPOCOTYL (LPH)* gene whose activity is indispensable for spatiotemporally correct expression of CYTOKININ INDEPENDENT-1 (CKI1), the sensor histidine kinase that activates MSP signaling. *lph* is a new allele of *HEME OXYGENASE 1 (HY1)* which encodes the key protein in the biosynthesis of phytochromobilin, a cofactor of the photoconvertible light receptors phytochromes. Following analysis confirmed light-dependent regulation of the pattern of *CKI1* expression and revealed that far-red light-induced downregulation of *CKI1* requires functional phytochrome A (phyA). phyA-regulated PHYTOCHROME INTERACTING FACTOR 3 (PIF3) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) TFs were found to bind the *CKI1* promoter, thus further supporting direct regulation of *CKI1* by phyA-mediated signaling. Attenuation of MSP signaling and reduced cytokinin sensitivity detected in both *lph/hy1-7* and *phyA phyB* loss-of-function mutant demonstrates the importance of functional light perception for cytokinin response, possibly via light-regulated *CKI1* expression. Finally, changes in the spatiotemporal specificity of *CKI1* expression in the *hy1-7* result into cytokinin-related developmental aberrations that were previously shown to be associated with CKI1 action. We propose that the light-dependent regulation of *CKI1* provides a plausible mechanistic link underlying the well-known interaction between light- and CK-controlled plant development.

*Supported by the European Regional Development Fund (Central European Institute of Technology project no. CZ.1.05/1.1.00/02.0068), the European Social Fund (CZ.1.07/2.3.00/20.0189) and the Czech Science Foundation (P501/11/1150 and 13-25280S).*

### P5-6 Cross talk between auxins, ethylene and nitric oxide during light-mediated greening and plastid development in de-etiolating tomato seedlings

**Luciano Freschi[1], Nielda Karla Gonçalves de Melo[1], Paulo Marcelo Rayner Oliveira[1], Diego Demarco[1], Lazaro Eustaquio Pereira Peres[2]**
*[1]Department of Botany, University of Sao Paulo (USP) – Institute of Biosciences, Sao Paulo, Brazil; [2]Department of Biological Sciences (LCB), University of Sao Paulo (USP) – ESALQ, Piracicaba, Brazil*

E-mail of presenting author: freschi@usp.br

The transition from etiolated to green seedlings involves the conversion of etioplasts into mature chloroplasts via a multifaceted light-driven process comprising multiple and tightly coordinated endogenous signaling networks. Plant hormones and other signaling molecules, such as nitric oxide (NO), are believed to play important roles in controlling the acquisition of these photomorphogenic traits. To investigate the involvement of auxins, ethylene and NO in the light-evoked greening and chloroplast development in tomato (*Solanum lycopersicum*), we have characterized the time course of plastidial differentiation, greening, and hormonal and NO fluctuations in photomorphogenic and hormonal mutant seedlings under continuous darkness or monochromatic red (RL) or blue light (BL). Moreover, transgenic seedlings carrying the synthetic auxin-responsive (*DR5*) and ethylene-responsive (*EBS*) promoters fused to the reporter GUS were also used to spatially and temporally localize the impacts of light on the tissue responsiveness to these hormones. A series of evidence indicated a mutual synergism between auxins and NO. (i) RL- or BL-induced greening and chloroplast differentiation temporally coincided with increases in both NO and auxins. (ii) Endogenous NO was drastically decreased and increased in de-etiolating seedlings of auxin-insensitive and auxin-hypersensitive tomato mutants, respectively. (ii) Exogenous NO completely rescued the auxin deficiency observed in the phytochrome-deficient *aurea* (*au*) mutant under RL. In contrast, a mutual antagonism between NO and ethylene was evidenced by a number of findings. (i) Whereas exogenous NO stimulated cotyledon greening, treatments with ethylene severally impaired light-induced greening. (ii) Ethylene-treated and ethylene-depleted de-etiolating seedlings presented decreased and increased NO levels, respectively. (iii) Exogenous NO drastically reduced ethylene emission in *au* seedlings under RL. Taken together, these data reveal that positive and negative feedback regulatory loops respectively orchestrate the auxin-NO and ethylene-NO interactions during the coordination of the tomato seedling transition from the etiolated state to photomorphogenic growth (Supported by FAPESP, CNPq and CAPES).

### P5-7 Aromatic cytokinin-like derivatives with significant biological activity in plant and animal cells

**Martin Hönig[1], Lucie Plíhalová[1], Jiří Voller[2], Karel Doležal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnical and Agricultural Research, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic; [2]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: mhonig90@gmail.com

Kinetin and benzylaminopurine (BAP) are best-known members of extenstive family of compounds called aromatic cytokinins. Both are well-known for their various biological activities, both in animal and plant cells. Aromatic cytokinins induce cell division, differentiation, morphogenesis and delay leaf senescence that makes them very useful in micropropagation techniques. Kinetin and its derivatives act often as antioxidants and particularly kinetin is able to delay age-related characteristics in human fibroblast cells. These properties could be and currently are utilized in anti-ageing skin care. Presented work deals with the preparation and study of aromatic cytokinin-like derivatives. New modified routes of preparation *via* nucleophilic substitution of 6-chlorpurine or 2,6-dichlorpurine core by appropriate amines was introduced and improved.These derivatives were screened in variety of bioassays in plants, such as *Amaranthus* caudatus betacyanin test, *Tobacco* callus biotest and detached *wheat* leaf senescence (chlorophyll retention) biotest. The above mentioned bioassays revealed cytokinin activity of prepared derivatives. Possible anticencer activity and cytotoxity on animal cells were studied on tumor cells lines K-562 and MCF7 and toxicity of the compounds was also studied on BJ human fibroblast cells. Biological activity of these derivatives was put into relation with their structure.

### P5-8 Identification of novel components of auxin-cytokinin cross-talk by transcriptome profiling

**Andrej Hurný[1], Candela Cuesta Moliner[1], Jerome Duclercq[2], Eva Benková[1]**
*[1]Institute of Science and Technology Austria, Institute of Science and Technology Austria, Klosterneuburg, Austria; [2] Department of Plant Systems Biology, VIB, Gent, Belgium)*

E-mail of presenting author: andrej.hurny@ist.ac.at
E-mail of corresponding author: eva.benkova@ist.ac.at

Lateral root organogenesis in *Arabidopsis* is governed by a complex network of hormonal regulations. Within all plant hormones controlling this process, auxin and cytokinin are demonstrated to be the key regulators of this developmental process. To investigate the molecular mechanisms of their hormonal crosstalk, genome-wide transcriptome profiling was taken as the main strategy. We aimed to identify genes at whose transcriptional regulation auxin and cytokinin pathways converge during early lateral root organogenesis. The *SYNERGISTIC AUXIN CYTOKININ1* (*SYAC1*) gene coding for a protein of unknown function was identified as a gene on which transcriptional regulation both auxin and cytokinin converge in synergistic manner. The motifs critical for the auxin-cytokinin regulation in *SYAC1* promoter were identified and we aim at characterization of the transcriptional apparatus mediating the synergistic auxin-cytokinin regulation of common target gene. In parallel a functional analyses are in progress to investigate its biological importance within the root developmental context.

### P5-9 Impact of ESR2 on auxin and cytokinin crosstalks during shoot regeneration

**Yoshihisa Ikeda[1], Ivona Kubalová[1]**
*[1]Molecular Biology, Palacky University in Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: yoshihisa.ikeda@upol.cz

A balance between the two phytohormones, auxin and cytokinin, is crucial to manipulate cell fate in an *in vitro* tissue culture. *Arabidopsis ESR2* (*ENHANCER OF SHOOT REGENERATION 2*), a member of AP2/ERF transcription factor family, exerts its transcription activation ability to promote cytokinin-independent shoot regeneration. Loss-of-function *esr2-2* mutant results in decreased *de novo* shoot development. Downstream genes directly up-regulated by ESR2 are shown to be involved in shoot apical meristem (SAM) formation, cell cycle control, and cytokinin and auxin signaling.
Toward a better understanding of the process of SAM initiation by *ESR2*, re-assessment of 51 target genes that were directly up-regulated by ESR2 was carried out. Up-regulation of non-canonical long-lived *IAA20* that lacks domain II and conserved Lys residue suggests an inhibitory cue of auxin primary signaling pathway. Along with direct up-regulation of cytokinin signaling inhibitor, *AHP6*, as well as degradation of active cytokinins by direct up-regulation of *CKX6* (*CYTOKININ OXIDASE/DEHYDROGENASE 6*), *ESR2* plays a role in modulating auxin and cytokinin signaling/metabolism pathways during *de novo* SAM organization in *Arabidopsis* tissue culture system.

### P5-10 Stabilisation of Arabidopsis cytokinin levels during Verticillium infection suppresses the fungi-induced symptomps and inhibits its proliferation in infected plants

**Jana Klásková[1], Michael Reusche[2], Karin Thole[2], Jekaterina Truskina[2], Ondřej Novák[3], Dennis Janz[2], Lukáš Spíchal[3], Miroslav Strnad[3], Volker Lipka[2], Thomas Teichmann[2]**
*[1]Department of Chemical Biology and Genetics, Centre of Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic; [2] Department of Plant Cell Biology, Albrecht-von-Haller-Institute, Georg-August-University Goettingen, Goettingen, Germany; [3]Department of Chemical Biology and Genetics, Laboratory of Growth Regulators and Institut of Experimental Botany AS CR, Centre of Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic*

E-mail of presenting author: jana.klaskova@upol.cz

*Verticillium longisporum* is a plant pathogenic fungi causing crop failures of crucifers family (*Arabidopsis*, *Brassica* plants). The soil-borne fungi colonizes plant root system, stem and leaves. Symptomps of the disease – "*Verticillium* wilt" are early flowering, leaf chloroses and stunting. Early senescence of *Verticillium* infected plants has been observed as a part of its infection strategy. Gene analysis of the senescence marker genes *SENESCENCE ASSOCIATED GENE12, SENESCENCE ASSOCIATED GENE13* and *WRKY53* revealed that the observed chloroses are a consequence of premature senescence triggered by *Verticillium* infection. Besides, the fungi also induces the expression of cytokinin oxidase genes and it causes decreased level of cytokinins in infected plants. Stabilisation of plant cytokinin levels during *Verticillium* infection by pharmacological and genetic approaches suppresses the *Verticillium*-induced symptomps and finally, it inhibits the fungal proliferation in infected *Arabidopsis* plants.

## P5-11 Do auxin and cytokinin cross-talk during pollen tube growth?

**Lidija Kovaleva[1], Alexander Voronkov[1], Igor Andreev[1]**
*[1]Russian Academy of Sciences, Timiryazev Institute of Plant Physiology, Moscow, Russian Federation)*

E-mail of presenting author: kovaleva_l@mail.ru

Pollen tube growth (PTG) is the best known example of highly polarized plant cell expansion. This process is known to require intensive exocytosis at the tip which is supported by dynamic cytoskeleton, vesicle trafficking, and functioning of ion transporters in pollen tube (PT) plasma membrane. Our studies carried out on two experimental systems, *in vitro* germinating male gametophyte and pollen-pistil of petunia, revealed an impact of phytohormones (ethylene, IAA, abscisic acid, gibberellins, cytokinin) on PTG which both *in vivo* and *in vitro* was accompanied by changes in their endogenous level. F-actin was found to be a putative phytohormone target. It was established that IAA stimulates PTG by increasing by 40% of the amount of F-actin in apical and subapical zones of PT indicating intensification of cytoplasm flow and vesicular transport. At the same time, an inhibition of PTG observed on the medium with latrunculin B was accompanied by the decrease in the amount of IAA to zero. In addition, the above stimulating action of IAA may be result of increased $H^+$-ATPase activity putatively mediated by $Ca^{2+}$ and generation of ROS by NADPH oxidase.Unlike IAA, cytokinin exhibited a pronounced inhibitory effect on PTG that was reflected in the decrease in the amount of F-actin along all PT length and the absence of any marked action on the $H^+$-ATPase. Results obtained allowed to conclude that IAA and cytokinin by means of their cross-talk are capable of involving in the regulation of PTG. However, the molecular mechanisms underlying the interaction between IAA and cytokinin during PTG remain unknown.

## P5-12 Probing the Unknowns in Cytokinin-Mediated Immune Defense in Arabidopsis with Systems Biology Approaches

**Meik Kunz[1], Muhammad Naseem[1], Thomas Dandekar[1]**
*[1]Department of Bioinformatics, University of Wuerzburg, Wuerzburg, Germany*

E-mail of presenting author: meik.kunz@uni-wuerzburg.de
E-mail of corresponding author: dandekar@biozentrum.uni-wuerzburg.de

Plant hormones involving cytokinin (CK), salicylic acid (SA), jasmonic acid (JA), ethylene (Et), and auxin, gibberellins, and abscisic acid (ABA) are small signaling molecules known to regulate almost every aspect of the plant life cycle including immune responses[1,2]. Moreover, CK regulates not only growth and development but also immunity and has the potential to modulate defense signaling mediated by SA and JA. Recently, enhanced CK level has been shown to increase plant resistance against pathogen infection[2,3,4,5]. However, the underlying mechanisms highlighting its implications in plant immunity are not well understood.
To identify hub points of immune interaction mediated by CK signaling upon pathogen infection in *Arabidopsis we* adapted systems biology approaches. High confidence Arabidopsis Protein-Protein Interaction networks[6] are mapped to changes in CK-mediated gene expression after treatment with *Pst* DC3000 (GSE6832.1) and *Hpa* Noco2[7]. Nodes of the cellular interactome enriched in immune functions were filtered out and their interacting partners reconstituted into sub-networks (method see reference 1). Based on different criteria such as topological parameters, gene expression and specific immunological relevance we identified functional hubs in our immune sub-networks. According to our analysis, de-repression of CK responses through the deletion of type-A ARRs promotes the SA pathway of resistance and points to a link between CK signaling and WRKY transcription factors in controlling immune dynamic in *Arabidopsis*. In our analysis, enhanced CK levels through external application modulate immune responses by activating JA pathway nodes. Taken together, our analyses identified hubs integrating functional modules as cross-linking agents between CK-mediated immune defense and pathways of resistance against pathogen infection in plants.

*(1) Naseem, Kunz et al. doi:10.4137/BBI.S13462*
*(2) Kunz et al, Biohelikon 2013*
*(3) Choi et al. doi: 10.1016/j.tplants.2011.03.003*
*(4) Naseem et al. doi: 10.1105/tpc.112.098335*
*(5) Navarro et al. PMID:18450451*
*(6) Szklarczyk et al. doi: 10.1093/nar/gkq973*
*(7) Argueso et al. doi: 10.1371/journal.pgen.1002448*

### P5-13 Regeneration of tomato (Lycopersicon esculentum Mill.): Somatic embryogenesis and Shoot Organogenesis from cotyledon, hypocotyl and mature embryo explants.

**Lachachi Sarra**[1], **Fyad-Lameche Fatima Zohra**[1]
[1]*Biology, Laboratory of genetic and plant breeding, Faculty of Science, Oran University, Oran, Algeria*

E-mail of presenting author: sara.lachachi@yahoo.fr

Tomato (*Lycopersicon esculentum* L.) one of the most important vegetables in the world. Numerous works research were devoted to *in vitro* culture either for mass production of elites genotypes or OGM. In this work the effects of genotype, explants and medium culture on organogenesis and somatic embryogenesis were studied in five cultivars of tomato (Rio Grande, Heinz, Agora, Top 48 and Aicha). The hypocotyl, cotyledon and mature embryo explants of seedlings *grown* on Murashige and skoog *(*MS) medium supplemented with 6-benzyladenine were subcultured on MS medium supplemented with BAP at different concentrations. Regeneration through Somatic embryogenesis and Shoot Organogenesis occurred in explants of all treatments, even on explants from seedlings grown on basal medium and subcultured to medium without growth regulators. The results showed that cotyledons produced the greatest number of organs and embryos on MS medium supplemented with BAP. Somatic embryos and shoots developed into complete plants on a medium lacking growth regulators. In vitro rooting was achieved on MS medium augmented with 0, 1 mg/l NAA (naphthalene acetic acid) in all the genotypes. Somatic embryos developed into complete plants on a medium lacking growth regulators

*Key words: Tomato, organogenesis, somatic embryogenesis, BAP, cotyledon, hypocotyl, mature embryo.*

### P5-14 Boron functions in meristem maintenance

**Paula McSteen**[1]
[1]*Division of Biological Sciences, University of Missouri, Columbia, Missouri, USA*

E-mail of presenting author: mcsteenp@missouri.edu

Auxin and cytokinin have been shown to play important roles in meristem function. By identifying mutants with defects in meristem formation, we have discovered additional molecules important in meristem function. The micronutrient boron (B) was shown to be essential for plant growth 90 years ago. B is known to crosslink pectin in the cell wall, but additional functions of B are still not well understood. We have characterized the *tassel-less1* (*tls1*) mutant in maize which has severe defects in inflorescence development, including absent or reduced tassels and aborted or ball-shaped ears, which are rescued by B supplementation. *tls1* encodes a major intrinsic protein in the aquaporin family, co-orthologous to the Arabidopsis B influx transporter *NIP5;1*. SEM and histological analyses indicate an early defect in the meristems of *tls1* mutants, highlighting the importance of B homeostasis in meristem function. In the root apical meristem, B deficiency has been shown to increase auxin and repress cytokinin responses. We are currently investigating the interaction of auxin and cytokinin in *tls1* meristems. This research suggests a previously under recognized area of crosstalk between essential nutrients and hormone signaling.

## P5-15 Molecular mechanisms of interaction between cellular expansion and cytokinin signaling

**Elena Pacifici[1], Riccardo Di Mambro[1], Rosangela Sozzani[2], Philip Benfey[3], Wolfgang Busch[4], Paolo Costantino[1], Sabrina Sabatini[1]**
*[1]Department of Biology and Biotechnology, University of Rome "Sapienza", Rome, Italy; [2]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, USA; [3]Department of Biology and Duke Center for Systems Biology, Duke University, Durham, USA; [4]Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria*

E-mail of presenting author: pacifici.elena@gmail.com

Plant development is predominantly a post-embryonic process ensured by the presence of stem cells that are located in specific structures named meristems. In the meristem stem cells undergo subsequent cycles of cell division, supporting plant indeterminate growth. In the *Arabidopsis thaliana* root apical meristem, these cells reaching the transition zone stop to divide and start to differentiate in specific root tissues. Cell differentiation input at the transition zone is controlled by cytokinin through the activation of the specific cytokinin-responsive transcription factor, *ARR1*. The switch between a meristematic cell and a differentiating cell is accompanied by changes in cell shape and size caused by the expansion process and a consequent cell wall remodelling. Cell expansion at the transition zone is controlled by the cytokinin-induced activity of specific members of the $\alpha$-expansins subfamily, involved in wall relaxation and activated by apoplastic acidification. Extracellular pH strongly depends on the plasma membrane $H^+$ ions flux driven by $H^+$-ATPase proton pumps. Our data show that the activity of two $H^+$-ATPase isoforms, AHA1 and AHA2, is necessary to induce cytokinin-mediated cell differentiation and expansion at the transition zone. Here we suggest novel components of the molecular mechanism that instructs meristematic cells to differentiate.

## P5-16 Cytokinin and Auxin Interactions in the Root Gravitropic Response in Arabidopsis

**Markéta Pernisová[1], Tomáš Prát[1], Jan Hejátko[1]**
*[1]FGPP, CEITEC, Masaryk University, Brno, Czech Republic)*

E-mail of presenting author: hejatko@sci.muni.cz

Root gravitropic response is regulated by differential auxin distribution and auxin-mediated regulation of cell elongation in the root tip. Fast changes in the intracellular localization of auxin efflux carriers from the PIN family in columella cells via BFA-sensitive transcytosis were demonstrated to be necessary for proper root gravitropic response. PIN proteins were previously shown to be controlled by cytokinins (CKs) and the importance of auxin/CKs interactions was demonstrated in the *de novo* organogenesis or lateral root formation.
To determine the potential role of CKs in the auxin regulated root gravitropic response, we described dynamics of root bending and PINs relocalization early after gravistimulation in transgenic lines with depleted endogenous CK levels (*Pro35S:AtCKX2* and *Pro35S:AtCKX3*) and in mutants in CK signalling pathway. In some of these lines we observed delayed gravitropic response. Several mutants in CK receptors (*ahk* lines) revealed aberrations in the auxin accumulation in columella cells, suggesting defects in the auxin transport machinery. Accordingly, the abundance or localization of PIN3 and PIN7 efflux carriers were affected in those lines, too. Using *in vivo* real-time imaging of PIN-GFP lines in the *CKX* overexpression and *ahk* mutant backgrounds we observed distinct dynamics of the PINs relocalization in columella cells early (5 min) after gravistimulation. Altogether, our results provide experimental evidence for the role of CK-regulated expression and localization of auxin transporters in the root gravitropic response. Importantly, our data suggest the yet unknown role of CKs in the PINs relocalization early after gravistimulation, representing thus novel mechanism of auxin/CK crosstalk.
*Supported by CEITEC CZ.1.05/1.1.00/02.0068, GACR P501/11/1150 and GACR 14-30004P.*

### P5-17 Study of cytokinin-interacting proteins in plants

**Vladimír Skalický[1], Radim Simerský[2], René Lenobel[2], Václav Mik[3], Ivo Chamrád[2], Miroslav Strnad[1]**

*[1]Laboratory of Growth Regulators, Palacky University & Institute of Experimental Botany ASCR, Olomouc, Czech Republic; [2]Department of Protein Biochemistry and Proteomics, Centre of the region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic; [3]Chemical Biology and Genetics, Centre of the region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

E-mail of presenting author: skalivlad@gmail.com

In this study we focused on isolation and identification of cytokinin-interacting proteins from plant tissue. A group of cytokinin binding protein (CBP) counts very few members which function is not still completely revealed. CBPs are probably involved in plant development. As a model material the suspension culture of *A. thaliana* ecotype L*er* was chosen. The first key step was a native proteins extraction which is needed for successful proceeding of the following affinity purification. Sepharose beads with isopentenyladenine ligands immobilized either on $C^2$ and $N^9$ were used for isolation of proteins. Purified proteins were subsequently separated by 1D SDS-PAGE, digested with trypsin and identified by MS/MS spectrometry. By this approach, several new potential cytokinin interacting macromolecules were revealed. Because the identified proteins play a roles in biological processes (e.g. carbohydrate, protein and lipid metabolism, response to stress and intracellular transport) influenced by cytokinin, these findings could contribute to understanding the mechanisms of physiological effects of these important plant hormones.

### P5-18 Fructose metabolism and auxin response

**Ofer Stein[1], David Granot[1]**

*[1]Department of Vegetable Research, Institute of Plant Sciences, Agricultural Research Organization, The Volcani Center, Bet-Dagan, Israel*

E-mail of presenting author: oferstein@gmail.com

Auxin has been known for a long time as a key regulator for many plant developmental processes including vascular development. Auxin can modulate sugar metabolism for the regulation of developmental processes such as cell divisions and expansion but may also be affected by the metabolic state and sugar levels. The major fructose phosphorylating enzyme in tomato (*FRK2*) is crucial for secondary vascular development and water conductance throughout the plant and the effects of *FRK2* suppression on auxin was evaluated.
PIN1 is a major auxin transporter. Down regulation of *FRK2* resulted a 2-fold increase in *PIN1* expression in mature stem and accumulation of PIN1 protein mainly in secondary xylem tissues.
Auxin response was also evaluated with the auxin synthetic promoter DR5-venus reporter. *FRK2* suppresion decreased DR5 expression in mature stem whereas no significant effects were observed in the shoot apical meristem (SAM). Suppression of *FRK2* also induced expression of other auxin response related genes such as Aux/IAAs (IAA3, 4, 10, 11, 17) and auxin response factor (ARF 7) in stems. These findings suggest that *FRK2* may affect vascular development at least partially by altering plant auxin response.

### P5-19 Methylated cytokinins: the secret weapon of the phytopathogen Rhodococcus fascians?

**Danny Vereecke[1], Adriana Cabecinhas[2], Jakub Koristka[3], Petr Tarkowski[3], Ine Pertry[4], Stefaan Werbrouck[1]**
*[1]Applied Biosciences, Ghent University, Ghent, Belgium; [2]ESTM, Polytechnic Institute of Leiria, Leiria, Portugal; [3]Centre of Region Haná for Biotechnological and Agricultural Research, Palacky University, Olomouc, Czech Republic; [4]Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium*

E-mail of presenting author: danny.vereecke@ugent.be

The actinomycete *Rhodococcus fascians* induces a disease, known as the leafy gall syndrome and characterised by the induction of multiple shoots, on a broad variety of dicotyledonous herbaceous plants. The main pathogenicity factor of the bacterium is the production of a mixture of 6 cytokinins (2-iP, *cZ*, *tZ* and their methylthio (MeS)-derivatives) that are produced by the genes of the *fas* operon which is located on a linear virulence plasmid pFiD188. To date, the only other organism known to carry a *fas* operon, is another phytopathogenic actinomycete, *Streptomyces turgidiscabies*. Interestingly, both bacteria also share two strongly related SAM-dependent methyltransferase genes (*mtr*) that are associated with their *fas* operons. Mutants in these *mtr*'s in *R. fascians* have lost all ability to provoke symptoms in their hosts. Importantly, the *mtr*'s are not involved in the production of the MeS-cytokinins, implying that other methylated cytokinins are imperative for disease induction. By determining the expression pattern of the *mtr* genes in *in vitro* and *in planta* grown *R. fascians* cells, we want to get insight into regulatory aspects of these genes. By analysing the cytokinin profiles of a set of *R. fascians* strains and of *S. turgidiscabies* fed with $^{14}$C-labelled SAM and $^{14}$C-labelled adenine, we are trying to uncover methylated cytokinins that might have stayed in the dark for such a long time.

### P5-20 Meristematic characteristics of Agrobacterium-mediated tumors on pea

**Alena Vinogradova[1], Maria Osipova[1], Ludmila Lutova[1]**
*[1]Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russian Federation*

E-mail of presenting author: alena_08_93@mail.ru

Proliferation and differentiation of cells are controlled by plant hormones and transcription factors. Disorders of these processes lead to the development of various abnormalities, such as tumor formation. Crown gall tumors caused by *Agrobacterium tumefaciens* are widespread disease. The T-DNA from the bacterial tumor-inducing (Ti) plasmid, carrying genes involved in auxin and cytokinin biosynthesis, integrates into plants genome, leading to tumor formation. The role of transcription factors in tumor development is not well studied. It can be assumed that the same set of transcription factors that control cell proliferation normally can be involved in tumor formation. In our work we use *Agrobacterium*-mediated tumors induced on pea (*Pisum sativum*) hypocotyls as a model. First, we analyzed the distribution of proliferating cells in tumors using fluorescent-labeled thymidine analogue 5-Ethynyl-2´-deoxyuridine (EdU) which is incorporated into the DNA of proliferating cells. We found that proliferating cells in tumors are clustered into meristematic foci. To study if known meristem regulators are involved in *Agrobacterium*-mediated tumor development, we analyzed tumor development on pea mutants *Pssym28* and *Pssym29* defected in CLAVATA (CLV) components, CLV2 and CLV1-like kinase, respectively. No influence of pea *PsSYM29* and *PsSYM28* genes on tumor diameter was found, suggesting these genes do not control the development of *Agrobacterium*-mediated tumor in pea.
Next, we have analyzed the expression of several meristem-specific genes (*WOX*, *KNOX*, *CLE*, *PLT*, *SHR*, *SCR* families) upon the development of *Agrobacterium*-mediated tumors. Interestingly, we found that expression of *WOX5* gene was increased in all tumor samples, suggesting that key root meristem regulator WOX5 is involved in *Agrobacterium*-mediated tumor development. Together, our results will provide a better understanding of mechanisms linking abnormal balance of plant hormones leading to tumor formation and meristem-specific transcription factors.

## P5-21 The role of Auxin and Cytokinins during the Activation of the Secondary Growth

**Riccardo Siligato[1], Kamil Růžička[1], Ari Pekka Mähönen[1]**
*[1]Department of Biosciences, Institute of Biotechnology / University of Helsinki, Helsinki, Finland)*

E-mail of presenting author: riccardo.siligato@helsinki.fi
E-mail of corresponding author: AriPekka.Mahonen@helsinki.fi

The secondary meristem cambium continuously produces phloem centrifugally and xylem centripetally, the latter forming the majority of biomass on Earth. Even though auxin, cytokinins, gibberellins and ethylene have been demonstrated to regulate the cambial activity, the corresponding molecular mechanisms remain poorly understood. Auxin and cytokinins signaling pathways, and the molecular crosstalk between them, drive and shape many aspects of plant development and physiology: embryo development, root meristem size maintenance, shoot stem-cell niche control, lateral root formation and primary pattering of the root vasculature.
Arabidopsis root primary vasculature shows diarch symmetry with a central xylem axis and two phloem poles, separated by two domains of intervening procambial cell files. In the procambium, high cytokinin signaling upregulates the expression and drives the lateral polarization of the auxin efflux carriers PINFORMED proteins, which pump auxin inside the future xylem axis generating a high auxin response. The procambial cells do not divide for several days after the formation of the primary pattern; the reactivation of (pro)cambial cell divisions, a process called secondary growth, starts approximately 5 days after germination in the root. However, the physiological and molecular mechanisms leading to the activation of secondary growth are unknown.
Our preliminary data show that procambial cells are competent for the activation of the secondary growth since the first days after germination, waiting for the proper cytokinin threshold-level to emerge. Cytokinin precursors from the phloem are symplistically translocated to the cambial cells and activated by the LONELY GUY genes. High auxin response overlap in the cambial cells with the high cytokinin response, and the latter is directly upregulating the former to reactivate the cell divisions in cambial cells. Further studies will unravel the molecular mechanisms underlying this new auxin-cytokinin crosstalk interaction.

## P5-22 A mechanistic framework for differential cell growth-guided apical hook formation

**Krzysztof Wabnik[1], Petra Žádníková[2], Qiang Zhu[1], Dominique Van der Straeten[3], Richard Smith[4], Dirk Inze[5], Jiri Friml[6], Przemyslaw Prusinkiewicz[7], Eva Benková[1]**
*[1]Developmental Plant Biology, IST Austria, Klosterneuburg, Austria; [2]Institute of Developmental Genetics, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich-Heine-University, Düsseldorf, Germany; [3]Department of Physiology, Ghent University, Ghent, Belgium; [4]Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Köln, Germany; [5]Plant Systems Biology, VIB, University of Ghent, Ghent, Belgium; [6]Cell and Developmental Plant Biology, IST Austria, Klosterneuburg, Austria; [7]Department of Computer Science, University of Calgary, Calgary, Canada,*

E-mail of presenting author: krzysztof.wabnik@ist.ac.at

Differential cell growth enables flexible plant development in the presence of environmental fluctuations, such as organ bending after light or gravity stimuli. A prominent example of such a differential cell growth in plants is the formation of apical hooks that protect the fragile shoot apical meristems when they penetrate the soil after germination. Asymmetric auxin distribution (auxin gradient) coordinates the apical hook development, and its formation largely depends on the concerted action of auxin transporters including PIN-FORMED (PIN) auxin efflux carriers. Although the role of auxin transporters in the apical hook development has been well studied, detailed mechanisms underlying formation and developmental translation of auxin gradients into the differential cell growth remain elusive. Here, we combined *in silico* and *in vivo* approaches to infer a minimal mechanism underlying auxin gradient-guided differential growth during establishment of apical hooks in the model plant *Arabidopsis thaliana*. Computer simulations based on experimental data demonstrate that the asymmetric PIN expression prevailing on the concave (inner) side of the apical hook establishes an auxin maximum in the epidermis on this side of the hook. We propose a mechanism that translates this maximum into differential growth, and thus curvature, of the hook that was experimentally validated. Our model assumes a tight interplay between the PIN-dependent polar auxin transport and auxin-mediated cell growth dynamics. Our combined experimental and modeling studies also reveal that the hook curvature degree is determined by both auxin-mediated differential cell growth and spatial pattern of cell proliferation that involve hormonal crosstalk.

# SESSION 6: RESPONSES TO ENVIRONMENT

## O6-1 Cytokinin-dependent modifications of source-sink relationships lead to enhanced crop stress tolerance

**Eduardo Blumwald**[1]
[1]*University of California, Dept of Plant Sciences, Davis, USA*

E-mail of presenting author: eblumwald@ucdavis.edu

Drought, the most prominent threat to agricultural production worldwide, accelerates leaf senescence, leading to a decrease in canopy size, loss in photosynthesis and reduced yields. We hypothesized that it may be possible to enhance drought tolerance by delaying drought-induced leaf senescence through the stress-induced synthesis of cytokinins. We generated monocot and dicot transgenic plants expressing an IPT (isopentenyltransferase) gene driven by $P_{SARK}$ (Senescence-Activated Receptor Kinase), a stress- and maturation-induced promoter. The regulated expression of *IPT* under the control of $P_{SARK}$ significantly improved drought tolerance in both laboratory and field conditions. Transgenic plants produced higher yields than wild-type plants in the field and the seeds from $P_{SARK}$*::IPT* plants were normal, indicating that the nutritional value of the transgenic seeds was not altered.
We used a multidisciplinary approach that combined genomics, proteomics, metabolomics and enzyme function analysis to identify and characterize cellular/biochemical components that regulate Carbon and Nitrogen metabolism in wild-type and transgenic $P_{SARK}$*::IPT* rice plants grown under water deficient conditions. In the transgenic plants, sucrose degradation-associated enzymatic activities remained constant, while sucrose synthesis activities were enhanced. The relatively high sucrose balance during stress allowed the transgenic plants the continued assimilation of carbon needed for energy generation during the stress episode. The stress-induced cytokinin production had a positive effect on nitrate uptake as well as on the expression of genes associated with primary N assimilation and N re-assimilation, enhanced higher protein synthesis and the strengthening of the transgenic plants sink capacity. Our results indicate a great potential for the development of crops with improved performance and yield in water-limited areas of the world.

## O6-2 Auxin signaling and metabolism during the clubroot disease of Arabidopsis: some evolutionary implications

**Jutta Ludwig-Müller**[1], **Sabine Jülke**[1], **Linda Jahn**[1], **Sabine Marschollek**[1]
[1]*Institute of Botany, Technische Universität Dresden, Dresden, Germany*

E-mail of presenting author: Jutta.Ludwig-Muelle@tu-dresden.de

The clubroot disease of Brassica species, among them *Arabidopsis thaliana*, is caused by the obligate biotrophic protist *Plasmodiophora brassicae*. The disease causes huge losses among brassica crops such as oilseed rape and cabbages, so to understand how the disease is regulated is of enormous interest. After colonization of the host, the roots are transformed into large gall like structures which gave the disease its name – clubroot. While it has been shown that the plant hormones auxin and cytokinin are responsible for disease development, the mechanism for signal transduction and regulation of, especially auxin homeostasis is less well understood. Here we have investigated how the auxin signal is perceived during clubroot and which genes could be possible targets. Among these genes expansins and the auxin conjugate synthetases of the GH3 family have been identified. Gene expression and promoter::reporter analysis confirm the specific transcriptional upregulation of the aforementioned genes during the clubroot disease. Mutant analysis for expansin and GH3 genes implicate further roles in the clubroot disease. Surprisingly, the clubroot pathogen *P. brassicae* also possesses genes with homology to plant hormone metabolism. Specifically, we have cloned a *GH3*-like gene from the protist and characterized it on the biochemical level. Although the function in the host plant is not known so far, we will discuss its possible evolution in comparison to other known plant GH3s. Altogether, this research contributes to the understanding how auxin conjugation might have been developed in different organisms.

### O6-3 Secrets of ancient patterns: The role of cytokinins in plant defense patterns

**Stefan Meldau[1], Martin Schäfer[2], Christoph Brütting[2], Radomira Vankova[3], Klaus Gase[2], Ian Thomas Baldwin[2]**
*[1]Molecular Physiology, KWS, Einbeck, Germany; [2]Molecular Ecology, Max-Planck-Institute for Chemical Ecology, Jena, Germany); [3]Molecular Physiology, KWS, Einbeck, Germany*

E-mail of presenting author: stefan.meldau@kws.com

The unequal distribution of defense metabolites in different tissues of plants (e.g. flowers, roots, stems, young and old leaves) has long been recognized by humans. An ecological explanation for these patterns is provided by the optimal defense theory. This theory predicts that the concentrations of defense metabolites in plant tissues are positively correlated with their fitness values and their probability of perceiving herbivore attack. Although it is well-known for many plant species that defenses are developmentally regulated, the underlying mechanisms remained unknown. Here we studied within-plant patterns of herbivory- and jasmonate-induced defenses in leaves of a native tobacco plant (*Nicotiana attenuata*). We found that cytokinin levels in leaves positively correlate with patterns of inducible defenses. Local scale manipulation of these ontogeny-dependent cytokinin levels was sufficient to deregulate inducible defenses and uncouple them from developmental regulation. Thus, our results provide evidence that the developmental regulation of growth hormones, such as cytokinins, plays a key role in establishing optimal defense patterns in plants.

### O6-4 The CRE1 cytokinin receptor pathway controls various environmental interactions in the *Medicago truncatula* legume root

**Carole Laffont[1], Thomas Rey[2], Olivier Andre[2], Mara Norevo[3], Stéphane Boivin[1], Mathias Brault[1], Frédéric Debelle[4], Paola Bonfante[5], Christophe Jacquet[2], Florian Frugier[1]**
*[1]Institut des Sciences du Végétal, Centre National de la Recherche Scientifique (CNRS), Gif/Yvette, France; [2]Laboratoire de Recherche en Sciences Végétales, CNRS / Université deToulouse, Castanet-Tolosan, France; [3] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Torino, Italy; [4] Laboratoire des Interactions Plantes-Microorganismes, CNRS-INRA, Castanet-Tolosan, France; [5] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Torino, France*

E-mail of presenting author: laffont@isv.cnrs-gif.fr

Cytokinin controls many aspects of plant development as well as environmental responses. However, the role of cytokinin in root systems able to respond not only to abiotic and biotic stresses but also symbiotic microorganisms, remains poorly understood. In legume crops, roots can develop two types of endosymbioses, with either nitrogen-fixing bacteria (Rhizobium) or mycorrhizal fungi (Gigaspora). As several cytokinin signaling genes are modulated in *Medicago truncatula* roots depending on different biotic and abiotic stress conditions, we also assessed potential involvement of the cytokinin receptor MtCRE1 (Cytokinin Response 1) pathway in abiotic and biotic stress responses. This pathway is first essential for initial cortical cell divisions leading to symbiotic nitrogen-fixing nodule organogenesis and also in mature nodule meristems (Plet et al, 2011). No significant arbuscular mycorrhizal (AM) phenotype was however detected in *cre1* mutant roots, indicating that MtCRE1 does not belong to the ancestral common symbiotic pathway shared by rhizobial and AM symbioses. When *cre1* roots were challenged with an abiotic stress (salt) or with a biotic stress (the *Aphanomyces euteiches* oomycete pathogen), a resistance phenotype was identified, correlated with the increased ability of *cre1* mutants to form lateral roots. This indicates that the MtCRE1 cytokinin pathway positively regulates infection by the root pathogen *Aphanomyces*, either directly or indirectly. Interestingly, a recent study showed that the *cre1* roots also have delayed symptoms in response to a bacterial pathogen, *Ralstonia solanaceraum* (Moreau et al., 2014).

*Plet et al. 2011 Plant J. 65(4):622-33. MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in Medicago truncatula.*
*Moreau et al. 2014 New Phytol. 201(4):1343-57 The symbiotic transcription factor MtEFD and cytokinins are positively acting in the Medicago truncatula and Ralstonia solanacearum pathogenic interaction.*

## O6-5 Role of plant hormones in flower development and fruit set under temperature stress

**Hagai Yasuor[1], Shiri Goldental[2], Irina Panizel[3], Naomi Ori[2], Asaph Aharoni[3]**
*[1]Vegetable and Field Crop Research, ARO, Gilat, Israel; [2]The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel; [3]Department of Plant Sciences, The Weizmann Institute of Science, Rehovot, Israel*

E-mail of presenting author: hagai@agri.gov.il

Abiotic stress results in damaged flowers and poor fruit set in vegetable and field crops. Reduced fruit set in tomato grown under high temperatures results from inhibited pollen development, pollen release, viability, germination and stigma elongation. Hormones play a pivotal role in most physiological processes in plants. The aim of this research was to elucidate the role of auxin and other plant hormones in flower development and fruit set in tomato under sub-optimal temperatures. We followed the distribution of the auxin response sensor DR5::VENUS (DR5) and analyzed the hormonal profile using the SPE purification method and LC-MRM-MS, during successive stages of flower and fruit development. DR5 expression was compared between wild type plants and *entire* mutant, mutated in a putative auxin response inhibitor. Auxin plays important roles in the transition from a vegetative shoot apical meristem into an inflorescence meristem and throughout flower and fruit development. High temperature reduced the expression of DR5 in developing anthers and stigmas. During early fruit development, DR5 expression was observed in various tissues in the fruit and embryo. A reduction in DR5 expression was observed in developing seeds, mainly in the placenta and integuments when fruit developed under either higher or lower then optimal temperatures (22/16ºC day/night). We are currently further characterizing the dynamics of DR5 expressiion in wild type and *entire* plants in optimal and suboptimal temperatures to further elucidate the role of auxin in flower and fruit devlopment under temperature stress. In addition, in order to elucidate possible interaction with other phytohormones we are developing a robust analytical method for hormone profiling, in which the analytes are concentrated from plant extracts and separated by chemical properties using consecutive SPE followed by analysis on UPLC-ESI-MS/MS.

## O6-6 Biochemical regulation in NaCl-stressed tomato plants treated with a novel inhibitor of cytokinin degradation (INCYDE)

**Adeyemi Oladapo Aremu[1], Johannes van Staden[1], Nqobile Andile Masondo[1], Taofik O. Sunmonu[1], Manoj G. Kulkarni[1], Marek Zatloukal[2], Lukáš Spichal[2], Karel Doležal[2]**
*[1]Research Centre for Plant Growth and Development, University of KwaZulu-Natal, Pietermaritzburg, South Africa; [2]Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, Czech Republic*

E-mail of presenting author: rcpgd@ukzn.ac.za

Globally, salinity stress remains a major agricultural and environmental challenge. The current study investigated the effect of 2-chloro-6-(3-methoxyphenyl)aminopurine (INCYDE = Inhibitor of cytokinin degradation) at 10 nM on growth and physiological status of NaCl-stressed (75, 100 and 150 mM) tomato seedlings. The resultant NaCl-induced decline in plant vigour was slightly reversed by both drenching and foliar application of INCYDE. Foliar application of INCYDE significantly increased the number of flowers in the control and 75 mM NaCl-supplemented plants, while drenching was more effective with the 150 mM NaCl stress treatment. Although INCYDE significantly increased the phenolic content in 75 mM NaCl seedlings, it had no remarkable stimulatory effect in the control and higher NaCl concentration-supplemented seedlings. Higher levels of malondialdehyde (MDA) associated with oxidative (lipid peroxidation) damage in leaf tissue which was evident in the presence of NaCl stress was significantly attenuated with the application of INCYDE. Similarly, activities of antioxidant enzymes (peroxidase, catalase and superoxide dismutase) were enhanced in the presence of INCYDE in the control and NaCl-stressed seedlings. Foliar application of INCYDE was generally more effective than drenching treatments. The current findings indicate the potential of INCYDE in protecting plants against the adverse effects of salinity stress.

### O6-7 Cytokinins can mediate tolerance to frost stress in *Arabis alpina* populations from the French Alps

**Evgueni Ananiev[1], Kalina Ananieva[2], Frédéric Laporte[3], Petre Dobrev[4], Václav Motyka[4], Miroslav Kamínek[4], Michel Herzog[3]**
*[1]Department of Plant Physiology, Faculty of Biology, University of Sofia, Sofia, Bulgaria); [2]Department of Photosynthesis, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria; [3]Laboratoire d'Ecologie Alpine (LECA), Université Joseph Fourier, Grenoble, France; [4]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, Prague, Czech Republic*

E-mail of presenting author: evgueni_ananiev@yahoo.com

Plants have evolved multiple mechanisms of tolerance as an adaptation to cold stress. These mechanisms can be mediated by a number of phytohormones including ABA, JA, SA and ethylene. However, data on the role of cytokinins (CKs) in cold tolerance are insufficient. The perennial plant *Arabis alpina* (Brassicaceae) was used as a model plant in a study on the involvement of CKs in the mechanisms of cold tolerance. Two populations of *Arabis alpina* were selected in the region of Grenoble: tolerant (T) and non-tolerant (NT) to frost collected in the French Alps (Col de Galibier) and in the mountain of Vercors, respectively. Plants were grown in a cultivation chamber for 10 weeks at 20°C under controlled conditions. Afterwards they were exposed to chilling stress (+4°C) for 4 days followed by freezing stress (-7°C) for 12h in darkness. Recovery from the frost stress was studied after return of plants to +4°C (4 days) and further to 20°C (4 days). The bioactive CKs (mainly iP and iPR) decreased in both T and NT after chilling stress and declined further at -7° C only in NT. The content of *cis*-zeatins was much higher compared to *trans*-zeatins and they showed the same trend of changes as the bioactive CKs in both *Arabis* populations. CK quantification data were in agreement with qRT-PCR analysis of CK biosynthesis genes indicating decreased *IPT3* together with increased *IPT2* transcription mainly in T population after both stresses. In addition, the mRNA level of *CKX7* (responsible in particular for *cis*-zeatin degradation) decreased in T after both stresses whereas in NT remained almost unchanged. Thus, the higher content of bioactive CKs and *cis*-zeatins in T population indicated involvement of CKs in the frost stress tolerance mechanisms of *A. alpina*.

### O6-8 Pils putative auxin transporters regulate apical hook development in *Arabidopsis thaliana*

**Chloe Beziat[1], Jürgen Kleine-Vehn[1]**
*[1]Applied genetics and cell biology, Bodenkultur universität, Wien, Austria*

E-mail of presenting author: chloe.beziat@yahoo.fr
E-mail of corresponding author: jkleinevehn@gmail.com

The plant hormone Auxin plays a crucial role in regulating plant growth and development, including root patterning, organ formation and growth responses to environmental stimuli. We identified novel putative auxin carriers (designated PILS proteins) that are localized at the endoplasmic reticulum and regulate intracellular auxin accumulation. PILS activity leads to conjugation-based reduction of free (active) auxin levels and subsequently limits cellular auxin signalling (Barbez *et al*., 2012). PILS activity controls cellular responsiveness to auxin and contributes to various developmental aspects. Here we show that PILS activity also controls differential growth regulation and reveal its involvement in environmentally controlled apical hook development. The apical hook of dark grown *Arabidopsis* seedlings is a structure, which develops soon after the germination to protect the meristem during the emergence of the seedling from the soil. Intercellular, polar auxin transport instructs asymmetric distribution and fluctuations of auxin, which is necessary to regulate the apical hook development. PILS putative auxin carriers show stage dependent expression during apical hook development. To monitor accurately the duration of the three sequential steps of hook development we used a real time system based on an infrared camera. Our study reveals that *PILS* gain-of-function have shorter maintenance and an early opening phase compared to the hook kinetics of wild type. On the contrary, *pils* loss-of-function mutants have a longer maintenance phase and delayed hook opening. These defects correlate with defective spatio-temporal auxin signalling during hook development. We also reveal that PILS proteins play a role in light induced morphological changes that we could correlate with a dynamics in the auxin signalling and in *PILS* expression in the hook zone. This data set indicates that PILS putative auxin carriers and, hence, presumably intracellular auxin transport processes are required to spatially control auxin responses during apical hook opening.

## O6-9 Shaded roots: a novel role of cytokinin in photomorphogenesis?

**Jan Novák[1], Martin Černý[1], Jana Zemánková[1], Břetislav Brzobohatý[1]**
*[1]Department of Molecular Biology and Radiobiology, Mendel University in Brno, Brno, Czech Republic*

E-mail of presenting author: novakhonza@atlas.cz
E-mail of corresponding author: brzoboha@ibp.cz

In land plants, roots have evolved, develop and function in darkness. However, analysis of seedling development in *in vitro* conditions employs cultivation in transparent Petri dishes resulting in unnatural exposure of roots to light. To evaluate influence of root illumination on seedling development and to get the first insight into underlying molecular processes, we have compared *Arabidopsis* seedling development in seedlings having roots fully exposed to light to those with strongly shaded roots. Morphometric analysis revealed that seedlings with shaded roots have shorter primary roots and elongated hypocotyls. Cellular analysis revealed that increased hypocotyl growth resulted from enhanced cell elongation. Next, we employed LC-MS approach to compare whole seedling proteome profiles in seedlings with shaded and illuminated roots. We succeeded in quantification of relative peptide abundances in 1209 proteins represented by more than 4200 peptides. The comparison after manual validation of MS spectra in Skyline software revealed 47 differentially regulated proteins quantified at 159 unique peptides. The largest group of these proteins is located in chloroplasts (28), followed by cytosol (14) and mitochondria (4). Functional analysis revealed that these proteins are involved, for example, in autotrophic $CO_2$-fixation and carbohydrate metabolism, nitrogen metabolism, amino acid metabolism and light absorption. Two differentially regulated proteins were then selected to validate their biological significance. Actin 2 was found up-regulated in seedlings with shaded roots. Actin 2 is reportedly involved in root hair elongation and its increased abundance corresponded with elongated root hairs in shaded roots. Among proteins down-regulated in seedlings with shaded roots, APT1 was selected for further analysis. APT1 catalyzes a reverse reaction to that of LOG and thereby inactivates cytokinins. Decrease in APT1 suggests an increase in active cytokinin pool in seedlings with shaded roots. A role of cytokinin in morphological alterations caused by root shading was proven in cytokinin receptor mutants.

## O6-10 Auxin feed-back on PIN3 polarity prevents gravitropic overbending in Arabidopsis hypocotyl

**Hana Rakusová[1], Hélène S. Robert[2], Jiří Friml[3]**
*[1]Department of Plant Systems Biology, VIB / Universiteit Gent, Gent, Belgium; [2]Developmental and Cell Biology of Plants, Central-European Technology Institute, Brno, Czech Republic; [3]Institute of Science and Technology Austria, (IST Austria), Klosterneuburg, Austria)*

E-mail of presenting author: harak@psb.vib-ugent.be

Tropic growth of plants responding to environmental variations belongs to the spectacular examples of plant adaptive development. Gravitropism is a growth reaction orienting plant's development in earth's gravitational field. Roots grow with the gravity vector, whereas shoots grow against it. Gravistimulus-induced redistribution of the phytohormone auxin was found to mediate tropic responses both in roots as in shoots. In both instances auxin transporting PIN3 re-localizes in response to gravistimulus correlating with redirection of auxin flow and preceding asymmetric auxin distribution. Regulations of PIN3 subcellular localization are thus important for execution of plant tropic responses.

The present study reveals the role of auxin feed-back on PIN3 polarity in termination of the gravity-induced bending. We show that PIN3 localization in the endodermal cells of hypocotyls is re-arranged by increased auxin concentration, and leads to PIN3 polarization to the inner side of endodermal cells. This would reduce the auxin accumulation at the bottom hypocotyl side and terminates the asymmetric cell elongation and bending. This PIN3 repolarization involves feed-back between auxin distribution and PIN3 intracellular trafficking as well as PIN3 protein stability. We also addressed the cellular and molecular mechanisms involved in auxin-dependent PIN3 relocation. We show that the established regulators of vesicle trafficking, cell polarity and auxin signaling are necessary for PIN3 repolarization and fine-tuning of hypocotyl gravitropism.

Our results suggest a mechanism to a previously unexplained phenomenon of bending response termination. We show how auxin, originally accumulating at lower side of the bending organ, repolarize PIN3 resulting in reduction of auxin asymmetry and in the termination of organ bending. Forward and reverse genetics strategies are deployed to identify novel components of the signalling cascade that link gravistimulation and auxin effect to PIN3 repolarisation in the *Arabidopsis* hypocotyl.

# POSTERS

## P6-1 Activation of cytokinin metabolism genes upon symbiotic nodule development

**Mahboobeh Azarakhsh[1], Maria Osipova[1], Ludmila Lutova[1]**
[1]*Department of genetics and biotechnology, St. Ptersburg state university, St. Petersburg, Russian Federation*

E-mail of presenting author: mahboobeazarakhsh@gmail.com

Cytokinin and auxin play fundamental roles in symbiotic nodule development. Legume plants with gain-of-function mutation in cytokinin receptor gene show spontaneous nodule development, whereas loss-of-function mutation in cytokinin receptor gene leads to nodulation deficient phenotype. Moreover, the activation of cytokinin signaling is essential for nodule development. Besides that, the inhibition of polar auxin transport in legumesinduces the formation of pseudonodules, and the expression of auxin transpoters is negatively regulated by cytokinin. So, the inhibition of polar auxin transport by cytokinin might contribute to the establishment of local auxin accumulation and finally nodule formation.
However, the reason of cytokinin signaling activation has not been found up to now. We hypothesized that cytokinin activation can be mediated by *IPT* or *LOG* genes involved in cytokinin biosynthesis and activation, respectively. We have identified the upregulation of individual members of the *IPT* and *LOG* families during nodule development. It is known that in shoot apical meristem KNOX transcription factors are responsible for the activation of cytokinin biosynthesis genes. We proposed that KNOX also might be involved in activation of cytokinin metabolism genes upon nodulation. We have analyzed the expression of different *KNOX* genes during different stages of nodule development. We found the activation of *KNOX3* expression in response to rhizobium inoculation and analyzed its local expression using pKNOX3:GUS construction. Based on our observation, at early stages *KNOX3* is expressed throughout nodule primordia, and at later stages its expression is observed in apical part of nodule, where nodule meristem is going to form, and also in pro-vascular bundle tissues. To study the role of KNOX3 transcription factor, we have constructed vector for KNOX3 overexpression. Together, our results contribute to understanding the mechanisms of nodule development and cytokinin activation upon nodulation.
*This work was supported by RFBR 14-04-00591, 13-04-02140 grants, NIR SPbGU 1.38.676.2013, HIII-5345.2012.4.*

## P6-2 Inhibition of growth and IAA biosynthesis in *Azospirillum brasilense* by L-amino acids

**Fabricio Cassán[1], Rivera Botia Diego Mauricio[1], Molina Romina[1]**
[1]*Laboratorio de Fisiología Vegetal y de la Interacción planta-bacteria, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina*

E-mail of presenting author: fcassan@exa.unrc.edu.ar

*Azospirillum* sp. is one of the most relevant PGPR genus studied worldwide in last decades. Indole-3-acetic acid (IAA) is probably the most important molecule produced by *Azospirillum brasilense* and it is considered to be the a key molecule during plant-bacteria interaction and plant growth promotion. In previous works, we tested the effect of several amino acids on *A. brasilense* behavior in chemically defined medium. Our hypothesis states that bacteria growth and IAA biosynthesis can be specifically regulated by different amino acids [L-phe, L-ileu, L-leu, L-val, L-ser and L-ala]. For this, *A. brasilense* Az39, the most extensively used strain for biofertilizer production in Argentina, was incubated in MMAB minimal medium supplemented with 100 µg.mL$^{-1}$ L-trp at 37 ºC and 180 rpm until reach exponential growth phase ($DO_{595}$ 0.6). Once done, culture was centrifuged, washed twice with sterile saline solution and fractionated in 10 mL capacity test tubes at 5 mL aliquots. The bacterial exposition to amino acids was made by individual addition of 100 µg.mL$^{-1}$ L-phe, L-ileu, L-leu, L-val, L-ser, L-ala solutions to each tube by triplicate. Distillated water and L-asp (no effect on Az39) were used as controls. The modified bacterial cultures were incubated during 4 hours in the same experimental conditions; after which were evaluated: IAA production (µg.mL$^{-1}$), biomass production ($DO_{595}$) and cellular viability (CFU.mL$^{-1}$). Our results shows that L-ileu, L-leu, L-val, and L-ala decreased the biomass production and significantly reduced the IAA biosynthesis ($P<0,05$); However, L-phe and L-ser although they affected the IAA production, did not affect bacterial growth. These results allow us to speculate about a new regulation mechanism for IAA biosynthesis in bacteria and to consider the implications of this mechanism in the rhizosphere by the production and secretion of amino acids by plant roots.

## P6-3 Down-regulation of cytokinin oxidase/dehydrogenase (CKX) activity is associated with the senescence-delaying effect of blue light in wheat leaves under shading stress

**Humberto Fabio Causin[1], Cintia Florencia Marchetti[1], David Zalabák[2], Petr Galuszka[2], Maria Šmehilová[2]**
*[1]Biodiversity and Experimental Biology, Faculty of Exact and Natural Sciences. University of Buenos Aires, C.A.B.A., Argentina; [2] Molecular Biology, Faculty of Science. Palacký University in Olomuc, Olomuc, Czech Republic*

E-mail of presenting author: ssvhfc@gmail.com

Suppression of blue light (BL) accelerates the senescence rate of wheat leaves exposed to shading. This phenomenon is correlated to an increase in oxidative stress symptoms, and previous data suggest that this response may involve changes in endogenous cytokinins (CKs) levels as well as $Ca^{2+}$ homeostasis. In order to better understand the interaction among light quality, CKs metabolism and $Ca^{2+}$ availability in the regulation of shade-induced senescence, detached leaf blades were exposed to either blue (B, high BL transmittance) or green (G, very low BL transmittance) Lee® filters, in absence or presence of 0.7 mM verapamil (a hyperpolarization-activated $Ca^{2+}$channels blocker) or 4.0 mM EGTA (a $Ca^{2+}$ chelator) at defined time points. Leaf samples were analyzed for changes in chlorophyll concentration, CKs content, cytokinin oxidase/dehydrogenase (CKX) activity and gene expression profile of *TaCKX1*. At 96 hrs after initiation of the treatments, BL transmittance significantly delayed senescence rate either in leaves continuously exposed to the B filter, as well as in leaves previously exposed (52 h) to the G filter. Nevertheless, this senescence-delaying effect of BL was partially suppressed when $Ca^{2+}$ availability was negatively affected. The concentration of active CKs markedly decreased at 24 hrs after initiation of the light treatments in all conditions tested, however leaves exposed to the B filter maintained a higher level of active CKs than the remaining treatments during most of the experimental period. CKX activity increased as senescence progressed, but it was up-regulated in absence of BL, or when BL was supplied along with EGTA or verapamil. Moreover, verapamil particularly enhanced the amount of TaCKX1 protein as revealed by Western blot analysis, suggesting that $Ca^{2+}$ movilization through hyperpolarization-activated channels is required for the down-regulation of leaf senescence as well as CKX1 by blue light.

## P6-4 Growth promotion of the seawater-irrigated halophyte *Salicornia bigelovii* by endophytic auxin-producing actinomycetes

**Khaled El-Tarabily[1], Abdulmajeed Alkhajeh[1]**
*[1]Department of Biology, Faculty of Science, 15551, University of United Arab Emirates, Al-Ain, United Arab Emirates*

E-mail of presenting author: ktarabily@uaeu.ac.ae

*Salicornia bigelovii* is a promising halophytic crop for saline soils, and can therefore contribute to arid-zone management. *S. bigelovii* has substantial economic value as a bioresource, in addition to its importance as a culinary product. Endophytic actinomycetes were isolated from *S. bigelovii* roots to evaluate their potential for plant growth promotion and investigate the mechanisms involved. Twenty-four endophytic isolates obtained from *S. bigelovii* roots were initially selected based on their ability to produce indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA) *in vitro*. An isolate of *Spirillosporaalbida* produced the highest levels of both IAA and IPYA. The addition of L-tryptophan (L-TRP) to broth inoculated with *S. albida* enhanced the production of IAA and IPYA several fold. Under greenhouse conditions using saline soils amended with or without L-TRP, *S. bigelovii* seedlings inoculated with *S. albida* exhibited significant increases in the fresh and dry weights, and lengths of roots and shoots. The growth promotion observed by *S. albida* was most pronounced in the presence of L-TRP treatment compared to the absence of L-TRP. This growth promotion was supported by the significant increases in the levels of *in planta* IAA and IPYA, compared with control plants. A mutant isolate of *S. albida* that was incapable of producing IAA *in vitro*, failed to increase the endogenous levels of IAA and IPYA and failed to promote plant growth, compared to plants inoculated with the wild type strain. The pure culture of *S. albida* was incapable of producing gibberellic acid, isopentenyl adenine, or zeatin indicating that the mechanism of growth promotion was likely to be due to IAA production. Our findings suggest that yields of *S. bigelovii* can be enhanced by the field application of this endophytic isolate. This study is the first to demonstrate the potential of endophytic actinomycetes to promote plant growth under saline conditions.

### P6-5 Enhancement of growth of *Salicornia bigelovii* seedlings by rhizosphere-competent plant growth regulators-producing marine actinomycetes

**Khaled El-Tarabily[1], Abdulmajeed Alkhajeh[1], Yaser Torky[1]**
*[1]Department of Biology, Faculty of Science, 15551., University of United Arab Emirates., Al-Ain, United Arab Emirates*

E-mail of presenting author: ktarabily@uaeu.ac.ae

Saline habitats cover a wide area of our planet and halophytes are increasingly being used for human benefits. *Salicornia bigelovii* is a promising halophyte found along the coastline of many countries including the United Arab Emirates. This study aimed to isolate and identify rhizosphere-competent plant growth promoting marine actinomycetes from *Salicornia* rhizosphere and to evaluate their potential as biological inoculants to promote the growth of *Salicornia* seedlings. Seventy-three actinomycete isolates were initially screened for their ability to colonize *Salicornia* roots *in vitro* and to be rhizosphere-competent under a naturally competitive environment. Nine promising rhizosphere-competent isolates were subsequently evaluated for their ability to produce *in vitro* plant growth regulators (PGRs) (auxins, cytokinins and polyamines), and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Three rhizosphere-competent isolates namely, *Streptomyces* sp., *Microbispora* sp. and *Pilimelia* sp. were selected based on their superior abilities to produce PGRs and ACC deaminase. Greenhouse experiments were conducted to investigate the effects of these three isolates, individually or in combination, on *Salicornia* seedlings growth and endogenous levels of auxins, cytokinins and polyamines in both roots and shoots. While the individual application of each strain resulted in a significant plant growth promotion compared to the control, the combination of the three isolates resulted in superior levels of plant growth promotion. This was evident from the significant increases in the levels of photosynthetic pigments, *in planta* auxins, cytokinins and polyamines and the significant reduction of the endogenous levels of ACC, in roots and shoots compared with control plants or plants grown in soils inoculated with only individual strain. These three isolates are considered to have the potential to perform as plant growth promoters for *Salicornia* production in nutrient impoverished soils in arid coastal areas. This study is the first report to indicate the potential of marine actinomycetes to promote *Salicornia* growth under saline conditions.

### P6-6 Improving cadmium tolerance using a cytokinin antagonist

**Markéta Gemrotová[1], Lucie Mikulíková[1], Miroslav Strnad[2], Lukáš Spíchal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic; [2]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Palacký University, Olomouc, Czech Republi*

E-mail of presenting author: marketagemrotova@gmail.com

Concentration of cadmium (Cd) in the environment has the increasing tendency. Due to the fact that Cd is accumulated in edible parts of plants including crops, it becomes the worldwide environmental and human health problem. Cd is phytotoxic for plant and interacts mainly with photosynthetic pigments, causes oxidative stress by generating free radicals and oxygen active species. As a consequence the plants have limited growth and form low biomass. Recent studies have demonstrated that cytokinin receptors play role in the stress response to abiotic stress as transgenic plants with low sensitivity to cytokinins were shown to be resistant to drought, cold, and high salinity. In our study we use chemical modulation of the cytokinin perception to achieve effects similar to the transgenic plants. Here we present results of the effect of PI-55, a cytokinin antagonist, on plants growing under Cd stress. PI-55 treated plants better tolerate Cd exposure and show reduced characteristics of intoxication. PI-55 prevented chlorophyll degradation, membrane damage and growth retardation even after a single application in a submicromolar concentration. Our observations indicate possible utilization of cytokinin antagonists in biofortification or phytoremediation processes.

*This work was supported by GA CR, 14-07418P and OP VK, CZ.1.07/2.3.00/20.0165*

## P6-7 High-throughput plant phenotyping facility in Palacký University in Olomouc

**Jan Humplík[1], Tomáš Fürst[2], Alexandra Husičková[3], Dušan Lazár[3], Miroslav Hýbl[3], Lukáš Spíchal[1]**
*[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic; [2]Department of Mathematical Analysis and Applications of Mathematics, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic; [3]Department Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University Olomouc, Olomouc, Czech Republic*

E-mail of presenting author: jan.humplik@upol.cz

Since 2013, the Centre of the Region Haná for Biotechnological and Agricultural Research in Palacký University has been equipped with two PlantScreen phenotyping systems by Photon Systems Instruments (Brno, Czech Republic). The instruments work as measuring systems for high throughput phenotyping and are located in climate chambers with LED illumination (max. 1000 uE) and controlled environment (10-40°C, 30-99% r.h.).
The first fully automated system with the capacity of 1200 standardized Arabidopsis pots or 480 culture multiwell plates in fixed positions employs a robotic arm for XYZ positioning. The arm is equipped with a chlorophyll fluorescence imaging system for measuring photosynthetic parameters, visual imaging system for analyzing leaf area and growth rate, and with a VIS-NIR hyperspectral imaging system for the evaluation of optical indices and parameters. The system is now being validated to be used either for high throughput phenotyping of *Arabidopsis* plants, or high throughput screenings of compound libraries in various plate-based bioassays.
The second system, equipped with roller conveyer, has the capacity for high throughput phenotyping of up to 640 Arabidopsis plants, cereals and other crops grown in standardized pots. The measuring cabinet contains an acclimation chamber for dark adaptation of plants coupled with an automated weighting and watering area. The cabinet is equipped with chlorophyll fluorescence imaging and visual imaging systems (top and 2 side views), thermoimaging to detect stomata openness and SWIR hyperspectral imaging to reveal water content. The operating software enables automatic data evaluation. Currently, the measuring protocols for phenotyping of *Arabidopsis thaliana* and pea (*Pisum sativum*) are being optimized and validated.

## P6-8 Source/sink changes in cotyledons infected by *Rhodococcus fascians*

**Paula Jameson[1], Pragathi Dhandapani[1], Jiancheng Song[1,2]**
*[1]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; [2]School of Life Sciences, Yantai University, Yantai, China*

E-mail of presenting author: paula.jameson@canterbury.ac.nz

Virulent strains of *Rhodococcus fascians* cause leafy galls following leaf inoculation, and multiple shoots following inoculation of germinating seeds. We used *Pisum sativum,* a natural host for this pathogen and two strains of *R. fascians* – one virulent and the other avirulent. Following inoculation simultaneously with seed imbibition, we observed that the cotyledons inoculated with the virulent strain remained intact throughout a 40-day experimental period. Furthermore, the cotyledons turned green and accumulated chlorophyll, whereas the cotyledons of control and peas inoculated with the avirulent strain shrivelled as the plants developed. Using RT-qPCR, we monitored the expression of cytokinin biosynthetic (*IPT*), activating (*LOG*) and degradation (*CKX*) gene family members of pea, and *RfIPT, RFLOG* and *RfCKX* from the virulent *R. fascians* strain, using primers that discriminated between plant and microbial genes. To assess changes in source/sink dynamics we monitored expression of transporter genes including some members of the *SUT, AAP* and *SWEET* gene families.
Four hours following imbibition in log-growth cultures of *R. fascians*, the virulent *R. fascians* was detected in the seed coat. Expression of the *RfIPT, RfLOG* and *RfCKX* genes was detected and the expression of the pea *IPT, LOG, CKX, RR* and transporter genes was elevated relative to the control cotyledons and cotyledons inoculated with the avirulent strain. By five days post-inoculation, when multiple shoots were apparent, expression of *RfCKX, RfLOG, PsLOG8, PsCKX3 & 4, PsRRs* and transporter genes was again elevated relative to control and peas inoculated with the avirulent strain. Subsequently, chlorophyll levels increased. Elevated PsRR and greening imply elevated cytokinin and, along with elevated transporters, are evidence indicating the establishment of the cotyledon as a nutrient source for virulent *R. fascians*.

## P6-9 Characterization and expression of the *BrEXLB1* gene and its promoter from *Brassica rapa* L.

**Soo In Lee[1], Panneerselvam Krishnamurthy[1], Jin A Kim[1], Mi-Jeong Jeong[1]**
*[1]Department of Agricultural Biotechnology, National Academy of Agricultural Science (NAAS), Suwon, South Korea*

E-mail of presenting author: silee@korea.kr

Expansins are unique plant cell wall proteins that possess the ability to induce immediately cell wall extension *in vitro* and cell expansion *in vivo*. The expansin superfamily comprises four distinct families: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB). There is experimental evidence that EXPA and EXPB proteins are required for cell expansion and developmental processes involving cell wall modification, whereas the exact functions of EXLA and EXLB remain unclear. To explore the regulation of expansin expression under phytohormones and heavy metal stresses, a novel expansin gene, *BrEXLB1*, and its upstream region were obtained from Chinese cabbage (*Brassica rapa* L.*)*. *BrEXLB1* encodes a cell wall protein of the expansin-like B-subgroup of the expansin family. The expression of *BrEXLB1* is regulated by exogenous phytohormones, NaCl, and heavy metals. Sequence analysis of its upstream region revealed the presence of several putative *cis*-acting elements, including phytohormone response, abiotic stress response, and tissue-specific elements. GUS activity under control of the *BrEXLB1* promoter allowed observation of *BrEXLB1* spatial and temporal expression patterns. The reporter construct indicated that *BrEXLB1* is induced by exogenous gibberellin and auxin, and inhibited by abscisic acid, $CdCl_2$ and PEG6000. Our results demonstrate that the *BrEXLB1* gene may be involved in the signal pathway of hormone regulation of plant responses to abiotic stresses.

## P6-10 Aldehyde oxidase in leaves of wheat, maize and soybean

**Tamara Li[1], Ultash Orazbayeva[1], Zere Spankulova[1]**
*[1]Laboratory of Cell Engineering, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan*

E-mail of presenting author: tamaralee05@gmail.com

Presumably drought tolerant medium-maturing wheat *Triticum aestivum* cvs. Severyanka, Alem, Miras, and as standard Kaz10; early-maturing soybean *Glycinemax*cvs. Ustya (Ukraine), K589109 (Russia), K583583 HMAS 84 (USA), and as standardAlmatywith vegetation period of 85-95 days; and early-maturing maize *Zea mays* from the world collection IKV-6 – Ukraine, Russia, IK65-1 – Ukraine, and as standard 05438 – Kazakhstan with vegetation period of 100-110 days were studied.
The conversion of the aldehyde to abscisic acid (ABA) occurs by the enzyme aldehyde oxidase (*AO*). *AO* presumably also involved in the biosynthesis of other plant hormone – indole- 3-acetic acid (IAA) via oxidation of the substrate indole -3- acetaldehyde. We have studied changes in the activity of *AO* in leaves of wheat, maize and soybeans under irrigation and drought stress to identify stress-signaling role of phytohormones ABA and IAA. ABA plays an important role in the regulation of gene expression as well as physiological responses during water stress. It is assumed that the adjustment in the content of IAA and ABA promote the incorporation of adaptive mechanisms that allow plants to survive in extreme conditions.
Two *AO* isoforms were detected by native PAGE in wheat leaves compared with maize developing only one *AO* band. *AO* activity in irrigated Kaz10 (standard) was the same level as in drought subjected plants, but for Severyanka, Alem and Miras *AO* activity expressed definitely higher under drought.
Determination of *AO* activity in maize leaves was conducted under irrigation and drought at the 3-5 leaves and 20 days before throwing panicle vegetation stages, most sensitive to water deficit on the 10th day of drought development. *AO* activity in maize was increased in stressed plants with display one band.
Determination of *AO* enzyme activity in leaves of soybean was conducted under irrigation and drought in two reproductive phases of vegetation, the most vulnerable to moisture deficit – at the flowering and grain filling stages – R1 and R5. Biochemical determination of the enzymatic activity of *AO* in soybeans revealed the development of two bands.
Soybean plants exhibited an enhanced adaptation mechanisms and tolerance to drought compared with wheat and maize.

### P6-11 New urea-type cytokinin analogues with unique anti-stress properties but low cytokinin activity

**Jaroslav Nisler[1], Marek Zatloukal[1], Miroslav Strnad[1], Karel Doležal[1], Lukáš Spíchal[1]**
*[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University & Institute of Experimental Botany, Academy of Sciences of Czech Republic, Olomouc, Czech Republic*

E-mail of presenting author: jaroslav.nisler@gmail.com

Urea-type synthetic cytokinins such as thidiazuron (TDZ) and CPPU are compounds with strong cytokinin properties widely used in agriculture. We have synthesized a range of new bis-substituted urea derivatives related to TDZ and CPPU and tested their cytokinin activity. Surprisingly, some compounds exhibited significantly higher activity than tZ and TDZ in wheat leaf senescence assay, although they had very low activity in other cytokinin bioassays including an *Arabidopsis* ARR5:GUS reporter gene assay. Compounds with such a strong anti-senescence activity have not been described in the literature yet. Selected derivatives were further tested for their potential to retard senescence of wheat plants grown under salinity, drought and heat stress conditions. Results of these experiments showed that the most active compounds delay stress-induced senescence at the whole plant level and prolong the photosynthetic lifespan of treated plants. In agriculture, stress-induced senescence has an enormous negative impact to grain and biomass yield. The synthesis of presented compounds is simple and cheap and therefore these compounds can serve as a tool for further investigation of the mechanism which contribute to the regulation of stress-induced senescence and find potential application in the care of crops as yield protectants.

### P6-12 Salt stress effects on phytohormone dynamics in salt-sensitive *Arabidopsis thaliana* and salt-tolerant *Thellungiella halophila*

**Sylva Prerostova[1], Petre Dobrev[1], Alena Gaudinova[1], Dagmar Prochazkova[1], Radomira Vankova[1]**
*[1]Institute of Experimental Botany, Academy of Science CR, Prague, Czech Republic*

E-mail of presenting author: prerostova@ueb.cas.cz

Salt stress affects plant metabolism, including the metabolism of phytohormones. Phytohormones play an important role in the plant stress responses. This study was focused on comparison of the response of two species with different strategies: the salt-sensitive model plant *Arabidopsis thaliana* and its close relative, salt-tolerant *Thellungiella halophila (salsuginea)*. Both plant species were grown in hydroponics. The levels of abscisic acid (ABA), auxin (indole-3-acetic acid, IAA) and cytokinin (CK) metabolites were screened in *Arabidopsis* or *Thellungiella* exposed for 1 week to NaCl in a range 2 – 150 mM, or 150 – 350 mM, respectively. Time course of the response was followed in the interval 15 min to 24 h. Strength of stress was evaluated by membrane stability index and by expression of stress-induced genes. Severe stress was imposed above 75 mM for *Arabidopsis* and above 225 mM for *Thellungiella*.
Moderate stress caused a modest elevation of the stress hormone ABA and its derivatives in both genotypes. ABA regulated water relations as well as stimulated defence pathways. During severe stress, plant responses differed: *Arabidopsis* gradually faded and died, starting from apices; while *Thellungiella* preferentially protected apices. The older leaves enhanced their senescence, probably due to salt accumulation. High salt concentration caused the rise of ABA in shoots after 4 h. Moreover in *Thellungiella* apices, ABA was transiently elevated after 30 minutes. Basal ABA levels were higher in *Thellungiella* than in *Arabidopsis*. IAA levels were higher, too. IAA decreased in heavily stressed roots in both genotypes, simultaneously with root growth suppression. Severe stress led to reduction of CKs, mostly *trans*-zeatin. This decrease was compensated by elevation of *cis*-zeatin (the cytokinin with low physiological activity), but such substitution was in balance only in *Thellungiella*.
*The work was supported by the Charles University in Prague, project GA UK No. 306714.*

### P6-13 Root enhancement in barley (*Hordeum vulgare* L.) by using CKX transgene technology

**Eswarayya Ramireddy[1], Heike Gnad[2], Jens Weyen[3], Thomas Schmülling[1]**
*[1]Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences (DCPS), Freie Universität Berlin, Berlin, Germany; [2]Saaten-Union Biotec GmbH, Gatersleben, Germany; [3]Saaten-Union Biotec GmbH, Leopoldshoehe, Germany*

E-mail of presenting author: eswar.ramireddy@fu-berlin.de
E-mail of corresponding author: thomas.schmuelling@fu-berlin.de

In recent times the plant root system trait has gained importance as a prominent and underutilized tool for designing strategies to improve plant growth and yield. Roots are important to plants for sensing and transporting of nutrients and water from the ground. Therefore, root system architecture (RSA) becomes a major target for attempts to increase tolerance to abiotic stresses such as nutrient deficiency, drought and salinity. We have investigated the role of cytokinin in regulating RSA in barley and tested strategies to achieve root enhancement. Cytokinin is known to be a negative regulator of primary root growth and lateral root formation. Consistently, lowering the cytokinin content by ectopic expression of cytokinin-degrading *CKX* genes specifically in roots causes increased primary root elongation and enhanced lateral root formation in dicots such as *Arabidopsis* and tobacco. Plants with an enhanced root system were more tolerant to drought and nutrient deficiency (Werner et al., Plant Cell 22: 3905-3920, 2010). We investigated whether applying *CKX* transgene technology to barley would have similar consequences for RSA in this monocot plants. To this end we first identified four root-specific promoters in rice and fused these to the *CKX1* and *CKX2* genes of *Arabidopsis*. More than 300 transgenic barley lines containing eight different promoter::*CKX* combinations were obtained by *Agrobacterium*-mediated gene transfer. Barley plants with a predominant transgene expression in roots were identified and an increased CKX enzyme activity measured. Transgenic plants were shown to form a 25-30% larger root system on dry weight basis in a hydroponic test system. The performance of plants with an enhanced root system under different environmental conditions will be investigated.

### P6-14 Influence of water deficit on IAA and ABA levels in a range of leaf tissues of *Guzmania monostachia* differing in competence for CAM expression

**Maria Aurineide Rodrigues[1], Paulo Tamaso Mioto[1], Leonardo Hamachi[1], Helenice Mercier[1]**
*[1]Botany Department, Universidade de Sao Paulo, sao paulo, Brazil*

E-mail of presenting author: auri@usp.br

The Crassulacean acid metabolism (CAM) is a photosynthetic pathway that confers an important ecophysiological adaptation for plants inhabiting places with scarce and/or seasonal water supply, such as the epiphytic niche. *Guzmania monostachia* is an epiphytic-tank bromeliad that can up-regulate CAM under water shortage. Since auxin and abscisic acid (ABA) often play critical roles in plant adaptive responses to environmental challenges, this study attempted to further investigate the potential participation of these hormones during the drought-induced CAM expression in *G. monostachia* leaves at different ontogenetic phases. The endogenous levels of indole-3-acetic acid (IAA) and ABA, the histological features, and some key components of the CAM machinery were comparatively studied in both apical and basal portions of the younger and older leaves from adult plants subjected to either daily watering or drought for 7 days. The results revealed that all chlorophyll-rich tissues displayed a drought-induced up-regulation of the metabolic parameters analyzed as indicative of the CAM cycle (*i.e.* nocturnal accumulation of organic acids and increased expression/activity of PEPC and MDH). However, only leaf tissues from older leaves showed significant reduction in the relative water content after 7 days of drought, indicating a possible water remobilization from older to younger leaves in the plant rosette. Accordingly, younger leaves of the drought-treated plants displayed the general trend of higher increases in all metabolic parameters indicative of CAM expression. Interestingly, conspicuous increments of both IAA and ABA levels were mostly observed in the drought-treated tissues from older leaves. These results suggest that IAA and ABA could participate as signaling molecules in this tissue-compartmented response that leads to a potential remobilization of essential resources from older to younger leaves along the *G. monostachia* rosette. This hypothetical mechanism might represent an important strategy for both fitness adjustments and survival of *G. monostachia*. Supported by FAPESP.

## P6-15 Distinct environmental triggers modulate auxin responses to shape root system architecture

**Michel Ruiz Rosquete[1], Jürgen Kleine-Vehn[1]**
*[1]Applied Genetics and Cell Biology, BOKU University of Vienna, Vienna, Austria*

E-mail of presenting author: michelrr1975@yahoo.es
E-mail of corresponding author: jkleinevehn@gmail.com

Most of current knowledge on how plants project their secondary roots network within the soil regards two factors: density and length. However, concerning soil coverage it appears obvious that growth direction is an essential parameter. Freshly emerged lateral roots (LRs) display a horizontal growth (stage I). In a subsequent phase (II) LRs bend to gravity and an initial gravitropic set-point angle (iGSA) is thus established. In stage III (plateau), LRs maintain a growth direction dictated by the iGSA until they enter a last phase (IV), ultimately characterized by an almost complete vertical orientation. Our group has recently uncovered an important role for the hormone auxin and its polar transport in the earliest establishment of LRs directional growth (iGSA) (Ruiz Rosquete *et al*., 2013). We now aim to identify which hormonal/environmental triggers might regulate the length/duration of the plateau phase and thus modify the radiality of the root system. The auxin machinery seems to be involved also here. Exogenous auxin shortens the plateau, a phenotype mirrored by auxin overexpressor lines. Interestingly, and in contrast with the robustness of the iGSA, the plateau length is sensitive to several environmental stresses. As an example, iron deficiency shortens the plateau phase. Here we show that this iron starvation-triggered re-orientation of LRs requires auxin biosynthesis, perception and transport. Our preliminary data thus suggest that 1) the role of auxin as a main "shaper" of lateral roots geometry is not confined to the initial gravitropic bending but it extends to the control of the length of the plateau phase, and 2) the plateau phase of lateral root development might constitute the first check-point for environmental stimuli (other than gravity) to re-shape the radial distribution of the root system, pressumably by impacting on one or several of the molecular players of the auxin-driven tropistic growth.

## P6-16 The cytokinin dependent hypocotyl response at decreased PPFD

**Gabriela Rotková[1], Břetislav Brzobohatý[1]**
*[1]Department of Molecular Biology and Radiobiology, Mendel University in Brno, Brno, Czech Republic*

E-mail of presenting author: gabcarot@gmail.com

Cytokinins as light are one of the master regulators of development. It is known, that plant response pathways for light and cytokinins are in interaction. Hypocotyl elongation is one of results of shade avoidance response to low photosynthetic photon flux density (PPFD). The role of several plant hormones (e.g. auxin, gibberellins) in regulating hypocotyl growth is already well described. Recently, by physiological experiments, it was revealed the involvement of cytokinin in the hypocotyl response to low PPFD. Now, we would like to uncover molecular mechanism of this process. We provided a wide spread transcriptomic analysis (qPCR – using hydrolysis probes) of genes involved in cytokinin metabolism and signaling (IPTs, CKXs, LOGs, N-transferases, O-transferases, AHKs, AHPs, ARRs-A, ARRs-B, APRRs, CRFs, PUPs) to understand which of them are responsible for hypocotyl answer to low light intensity. Moreover, we used wild-type or mutant *Arabidopsis thaliana* plants grown under decreased (20 μmol $m^{-2}$ $s^{-1}$) light intensity to uncover the interaction between light pathway, cytokinins and other plant hormones. On the basis of this experiment we chose candidate genes and in order to clarify the role of cytokinin in shade avoidance response we will characterize the phenotype of their mutants.
*The work was supported by OP Education for Competitiveness (European Social Fund and the state budget of the Czech republic) CZ.1.07/2.3.00/30.0017 Postdocs in Biological Sciences at MENDELU and the project CEITEC – Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) from the ERDF.*

### P6-17 Auxin homeostasis in *Brassica rapa* as a mechanism of plant stress response

**Branka Salopek Sondi[1], Iva Pavlović[1], Dunja Šamec[1], Ana Smolko[1], Snježana Mihaljević[1], Ivana Šola[2], Gordana Rusak[2], Jutta Ludwig-Müller[3],**
*[1]Department for Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; [2]Division of Biology, Faculty of Science, Zagreb, Croatia; [3]Institute for Botany, Technische Universität Dresden, Dresden, Germany*

E-mail of presenting author: salopek@irb.hr

Auxin levels and the regulation of reversible auxin conjugation as a mechanism of auxin homeostasis has been investigated in Chinese cabagge (*Brassica rapa* L. ssp. pekinensis) upon biotic stress caused by Cucumber mosaic virus containing satellite RNA (CMVsat) infection and abiotic stress (salt stress, drought) as well as tretments with stress hormones (SA, JA, ABA). To detect the physiological state of plants and their ability to integrate given stress conditions, a set of biochemical markers (glutathione level, antioxidant enzymes activity, carbonyl content) was analysed. Endogenous free and total IAA, and ABA were analysed by GC-MS. The levels of auxin were significantly affected by biotic and abiotic stresses as well as treatments with stress hormones. All stress conditions caused perturbation of IAA levels with a tendency of increasing the IAA conjugate level. Among treatments with stress hormones, JA caused the most significant increase of free and conjugated IAA in comparison to others. Endogenous ABA was significantly higher under both abiotic stress conditions in comparison to the control showing a positive correlation with auxin levels. Results of gene expression experiments (qRT-PCR) showed an increased transcript level of auxin amidohydrolase *BrIAR3* upon abiotic stress as well as after treatment with JA. The results implicated an important role of auxin and interactions with stress hormones in the plant stress response.

### P6-18 Proteolysis of a cytokinin-activating enzyme protects *Mycobacterium tuberculosis* against nitric oxide

**Marie Samanovic[1], Shengjiang Tu[2], Ondrej Novak[3], Lakshminarayan Iyer[4], Fiona McAllister[5], L. Aravind[4], Steven Gygi[5], Stevan Hubbard[6], Miroslav Strnad[3], Heran Darwin[1]**
*[1]Microbiology, NYU School of Medicine, New York, USA; [2]Biochemistry and Molecular Pharmacology, NYU School of Medicine, New York, USA; [3]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Palacký University, Olmouc, Czech Republic; [4]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, USA; [5]Cell Biology, Harvard Medical School, Boston, USA, [6]Biochemistry and Molecular Pharmacology and The Skirball Institute, NYU School of Medicine, New York, USA*

E-mail of presenting author: marie.samanovic@nyumc.org

*Mycobacterium tuberculosis* is one of the most devastating microbial agents, as it infects nearly one-third of the world's population and kills nearly two million people annually. Proteasome activity protects *Mycobacterium tuberculosis* (*Mtb*) from elimination by host-produced nitric oxide (NO) via a previously uncharacterized mechanism. To elucidate the link between proteolysis and NO-resistance, we screened for suppressors of NO hypersensitivity in a *Mycobacterium* proteasomal ATPase (*mpa*) mutant and identified two independent mutations in Rv1205. We determined that Rv1205 is an *Mtb* proteasome substrate with similar activity to LONELY GUY, a plant enzyme involved in the synthesis of a group of phytohormones called cytokinins. Cytokinins play crucial roles in plant development and have recently emerged as major factors in microbe-plant interactions. We found *Mtb*, a human-exclusive pathogen, secretes cytokinins. Furthermore, the intracellular accumulation of aldehydes, which are breakdown products of cytokinins, is likely responsible for the sensitization of proteasomal degradation mutants to NO. Importantly, disruption of Rv1205 partially rescues the attenuated phenotype of an *mpa* mutant in mice. Thus, the accumulation of one protein, rather than numerous damaged proteins, is sufficient to sensitize *Mtb* to NO and attenuate bacterial growth *in vivo*.

### P6-19 Plant hormone control of cold stress responses in wheat

**Radomíra Vaňková[1], Klára Kosová[2], Petre Dobrev[3], Ondřej Novák[3], Václav Motyka[3], Alena Gaudinová[3], Sylva Prerostová[4], Pavel Vítámvás[2], Ilja Tom Prášil[2]**
*[1]Laboratory of Hormonal regulations in Plants, Institute of Experimental Botany AS CR, Prague, Czech Republic; [2]Crop Research Institute, Prague, Czech Republic; [3]Institute of Experimental Botany AS CR, Prague, Czech Republic; [4]Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic,*

E-mail of presenting author: vankova@ueb.cas.cz

Cold stress response is regulated in a dynamic way by a complex phytohormone cross-talk. The early response to cold (alarm phase) is associated with growth suppression, accompanied by a down-regulation of the pool as well as of signal transduction of cytokinins, gibberellins and auxin. This response is faster and stronger in winter wheat lines in comparison with the spring ones. The response of leaves, directly exposed to cold, is faster than that of crowns. Cold exposure causes a decrease in hydraulic conductivity of roots, which results in a rapid increase of water saturation deficit. Water status stabilization is controlled by abscisic acid, exhibiting transient up-regulation. Simultaneously, an accumulation of protective proteins (especially dehydrins) and elevation of the frost tolerance is initiated. The following acclimation phase is characteristic by plant adaptation to low temperature. An elevation of growth-promoting hormones (cytokinins, auxin and gibberellins) indicates an adjustment of plant metabolism to growth and development under low temperatures. The onset of cytokinin increase coincides with the minimum of cytokinin oxidase/dehydrogenase activity. These changes are more rapid in spring wheat lines. Abscisic acid decrease is accompanied by a gradual increase of the other stress hormones, especially salicylic acid and jasmonic acid. During resistance phase, the winter cultivars exhibit further elevation of frost tolerance, which is associated with a decline in active cytokinins and auxin. This down-regulation is not observed in spring lines, which are not able to further increase their frost tolerance. The onset of the reproductive development is characteristic by a transient maximum of active cytokinins (especially of *cis*-zeatin riboside) as well as a decrease in frost tolerance and dehydrin levels. This developmental switch is delayed in winter lines until the fulfilment of their vernalization requirement.
*Acknowledgement: This work was supported by the Czech Science Foundation, project no. 522/09/2058 and 506/11/0774.*

### P6-20 The positive effects of vermicomposts and vermicompost tea on plant growth are attributed to the presence of cytokinins

**Jean W. H. Yong[1], Swee Ngin Tan[2], Wei San Wong[1], Hong Zhang[1], Liya Ge[3], Tiffany S. W. Tow[2], Xin Chen[4]**
*[1]Science, Singapore University of Technology and Design, Singapore, Singapore; [2]Natural Sciences & Science Education Academic Group, Nanyang Technological University, Singapore, Singapore; [3]Nanyang Environment & Water Research Institute, Nanyang Technological University, Singapore, Singapore; [4]College of Life Science, Zhejiang University, Hangzhou, China*

E-mail of presenting author: jyong@sutd.edu.sg

Earthworms produced rich organic fertilizers called vermicomposts containing mineral nutrients and anecdotal evidence of biologically active phytohormones. Aqueous extracts of vermicomposts, namely vermicompost tea (VT), are known to have positive effects on plant growth but the putative substance(s) had never been unequivocally characterized. It was postulated that trace amounts of phytohormones in vermicomposts or its VT are beneficial for plant growth and development. After a series of screening exercises involving LC-MS/MS after solid-phase extraction or ultrasound-assisted extraction, we reported the presence of cytokinins (CKs) in VT: namely trans-Zeatin (tZ), $N^6$-Isopentenyladenine (iP) and $N^6$-Isopentenyladenosine (iPR). We postulated that iP is a good reflection of the microbial origin for CKs present in the VT due to its importance in CK biosynthesis pathways and high abundance provided by microorganisms. The agronomic performance of the test plants, growing on substrates with varying levels of vermicomposts or its VT, is discussed in relation to the presence of CKs.

## P6-21 Modified perception of light affects cytokinin metabolism in *Arabidopsis thaliana*

**Eva Žižková[1], Přemysl Souček[2], Klára Hoyerová[1], Pavel Mazura[2], Lucie Doležálková[3], Miroslav Kamínek[1], Jiří Malbeck[4], Petre Dobrev[1], Břetislav Brzobohatý[2], Václav Motyka[1]**

*[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Prague, Czech Republic; [2]CEITEC Mendel University, Mendel University in Brno, Brno, Czech Republic; [3]Faculty of Science, Charles University in Prague, Prague, Czech Republic; [4]Laboratory of Mass Spectrometry, Institute of Experimental Botany AS CR, Prague, Czech Republic*

E-mail of presenting author: zizkova@ueb.cas.cz
E-mail of corresponding author: vmotyka@ueb.cas.cz

Daily light/dark cycle together with functioning of phytohormones cytokinins (CKs) affect numerous plant developmental processes. For instance, light and CKs influence chloroplast and leaf development, delay senescence and flowering, inhibit hypocotyl growth elongation and control expression and translation of some light-regulated genes.
We have shown that light signal is an important input for modulating some CK-related genes and endogenous CK levels in *Arabidopsis* plants. The complex diurnal expression profiles of CK-biosynthetic genes (AtIPT1 – AtIPT9) of plants grown under different cultivation conditions (hydropony, soil, MS agar medium) and sampled at different age indicated a strong dependence of *AtIPT1* and *AtIPT5* on light/dark phase in shoots. In contrast, no diurnal oscillation of *AtIPT* transcript levels was recorded in roots. Although the content of endogenous CKs was not constant in plants and varied during a day, no statistically significant correlation between light/ dark cycle and oscillation in cytokinin levels both in shoots and roots was evaluated.
We used *Arabidopsis thaliana* mutants with disrupted perception of light to decipher by which regions of light spectra CK metabolism is regulated. Screening of *Arabidopsis* photoreceptor mutants (12 days old) sensitive to red (*phyA*, *phyB* and *phyAphyB*) and blue (*hy1*, *hy2*, *hy5* and *photA*) light showed that light quality affects plant growth and CK status.
*Supported by the Czech Science Foundation project P506/11/0774.*

# LATE ABSTRACTS

## PL-1 Cytokinins and organ source-sink relationships in plants: associations with microorganisms

**R.J.N. Emery[1,3], Anna Kisiala[1,4], E.N. Morrison[3], B.J. Saville[2,3]**
*[1]Biology Department; [2]Forensic Science; [3]Environmental and Life Sciences, Trent University, Peterborough, Ontario, Canada K9J 5Z9; [4]University of Technology and Life Sciences, Bydgoszcz, Poland*

E-mail of presenting author: nemery@trentu.ca

Cytokinins (CKs) are a group of phytohormones that control numerous developmental processes and are highly regulated within plants. Plant CK profiles differ depending on their developmental stage and environmental conditions and orchestrate growth among organs through their effects on cell division and partitioning of assimilates. Many recent studies have shown that, beyond plants, CK production is wide-spread among microorganisms, regardless of whether they interact with plants. However, it appears that microbial CK composition often reflects the microbe's ability to manipulate plant metabolism depending on the type of interaction. Our lab has compared CK metabolites of three groups of microorganisms, beneficial Pink Pigmented Facultative Methylotrophs (PPFMs), symbiotic Rhizobia and fungi with different modes of nutrition, these CK profiles will be presented. Detailed LC-MS/MS analysis of 48 strains of PPFMs originating from a variety of sources, revealed that these environmentally ubiquitous symbiotic bacteria cultured *in vitro*, secrete high levels of the most active CK fraction – free base Zeatin (up to 82.16pmol/mL of trans-Zeatin and 146 pmol/mL of cis-Zeatin). Zeatin is known to stimulate plant cell division which results in emission of methanol consequently utilized by *Methylobacteria* as a source of carbon. By contrast, CK profiles of symbiotic Rhizobia, consist mainly of methylthiols (often more than 80% of total CKs), which are abundant in microorganisms, but not known to be active CK forms in plants. This may reflect the different types of plant interactions between PPFMs, which results in general growth stimulation, and rhizobia, which are involved in nodulation. Finally, CK profiling of 20 temperate forest fungi, with differing modes of nutrition, revealed that specific CKs were present in all fungi sampled independent of their mode of nutrition (plant or non-plant interacting). CK profiles of these three groups of microorganisms will discussed along with their probable impact on plant growth and development.

## PL-2 Using automated high-throughput phenotyping to quantify and visualize early stress responses in plants

**Klara Panzarova-Simkova[1], Zuzana Benedikty[1], Martin Trtilek[1]**
*[1]Photon Systems Instruments Ltd., Drasov, Czech Republic.*

E-mail of presenting author: panzarova@psi.cz

Non-invasive capturing and interpreting of plant structural and functional phenotypes in controlled or dynamically changing environment is long-standing and necessary requirement for genetic and physiological research by crop breeders, agricultural industry, and academia. To sustain global food security the major challenge global agriculture and plant biology is nowadays facing is identification of new high-yielding genotypes of agricultural crops that are adapted to our future climate. The use of automated high-throughput phenotyping approaches offers the tool for accelerating crop breeding progress by linking the selected gene function with phenotypic traits in relevant environment.
To quantify and visualise dynamically signatures that might be informative of plant development, physiological status, and performance Photon Systems Instruments (PSI) developed a range of PlantScreen™, plant phenotyping platforms for the greenhouse, growth room and field. These incorporate imaging technologies for RGB and morphometric analysis, thermal analysis, hyperspectral analysis and, critically, in-depth analysis of chlorophyll fluorescence kinetics. The latter technique, as a rapid tool for monitoring photosynthetic processes, is key to the identification of early onset of stress, and recovery from stress after amelioration.
Here we used the PlantScreen™ platform to quantify and visualise early plant response to stress by analysing plant structure, growth, and physiological status after application of glyphosate, nonselective, broad-spectrum, postemergence herbicide. The herbicide glyphosate reduces plant growth and subsequently causes plant death by inhibiting the biosynthesis of aromatic amino acids. The aim of this study was to quantitatively investigate the time course of glyphosate mode of action in *Arabidopsis thaliana* and identify signatures informative of early onset of stress by using PlantScreen™ hyperspectral image analysis, morphometric analysis and in-depth analysis of chlorophyll fluorescence kinetics. These signatures can be used to quantify early effect of the herbicide prior any visual damage on plants can be observed by eye.
Our results demonstrate that already 3 hours after glyphosate application fluorescence decline ration (Rfd) used to asses plant vilality is reduced, whereas maximum photosynthetic efficiency (Fv/Fm) of the plants is affected only 6 hours after spraying. Normalized Difference Vegetation Index (NDVI), analysed by hyperspectral imaging, is an important indicator of chlorophyll content in plants. NDVI was reduced around 13 hours after glyphosate application, which is around 10 hours before any visual damage of the plants can be observed. Further we show that as result of glyphosate application growth dynamics of the plants is impaired within first 20 hours after spraying. We demonstrate the suitability of the PlantScreen™ platform for non-invasive high-resolution investigation of changes in plant performance after glyphosate application, as an example of stress treatment.

### PL-3 Rhamnosylated flavonols are involved in modifying plant development and auxin distribution

**Christoph Ringli, Sanae Errafi, Benjamin Kuhn, Rahel Bucher, Petre Dobrev, Laurent Bigler, Markus Geisler, Eva Zazimalova**

Flavonols are secondary metabolites with diverse functions in UV protection, cell-cell communication, and general plant (cell) growth processes. The Arabidopsis *rol1-2* (*repressor of lrx1-2*) mutant is defective in a rhamnose synthase, which induces a change in the accumulation profile of flavonol glycosides. The flavonols in *rol1-2* induce the development of hyponastic cotyledons and brick-shaped pavement cells, and blocking flavonol accumulation suppresses these *rol1-2* shoot phenotypes. Furthermore, the flavonols in the *rol1-2* mutant change auxin distribution and -transport.

In an EMS mutant screen, the flavonol-specific 7-rhamnosyltransferase (*7rt; ugt89c1*) was identified as a suppressor of the *rol1-2* shoot growth phenotype**.** Also, the auxin distribution defect of the *rol1-2* mutant is alleviated in the *rol1-2 7rt* double mutant. Together, this suggests that rhamnosylated flavonols are involved in inducing the *rol1-2* phenotypes. Further analyses, however, suggest that the *7rt* mutation causes changes in plant development that are independent of flavonols, raising the question whether the enzyme *7RT* (UGT89C1) is indeed specific for flavonols or rather has an addition, so far unknown function.

### PL-4 Does auxin involved in regulation of $H^+$-pump activity at transcription level?

**M. Shishova[1], Ju. Prokopieva[1], T. Chen[1], V. Yemelyanov[1]**
*[1]Saint-Petersburg State University, Saint-Petersburg, Russia*

E-mail of presenting author: mshishova@mail.ru

Cell elongation is a unique process for plant cell and requires plant hormone auxin to be triggered on. Nowadays a huge amount of data is accumulated which reveals a great importance of the plasma membrane $H^+$-ATPase in realization of auxin-induced cell enlargement. There are no clear evidences about the direct hormone action on the enzyme activity, thus a complicated puzzle of possible mechanisms required for $H^+$-pump activation at plasma membrane is still under the focus of different investigations. We showed a shift in the patterns of plasma membrane $H^+$-ATPase genes expression during elongation of Arabidopsis seedling with and without the hormone treatment. Besides the well-known role of $H^+$-ATPase in the process of extension growth this enzyme also plays a crucial role in the early transduction events after auxin addition. The primary auxin-induced cytosol acidification reflects cell sensitivity to the auxin. Thus we suggested a scheme of non-linear shift in $H^+$-ATPase activity regulated at the expression level and controlled by the auxin during elongation.

## PL-5 *Arabidopsis JAGGED LATERAL ORGANS (JLO)* sensitize plants to auxin

**Petra Žádníková[1], Madlen I. Rast[1,2], Rüdiger Simon[1]**
*[1]Institut of Developmental Genetics, Cluster of Excellence on Plant Sciences (CEPLAS) Heinrich-Heine-Universität, Universitätstrasse 1, 40225 Düsseldorf, Germany; [2] Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Köln, Germany*

E-mail of presenting author: Petra.Zadnikova@uni-duesseldorf.de

Auxin is the most studied plant hormone and has been shown to be involved in controlling fundamental aspects of plant development, such as cell fate determination, cell division and cell polarity. Althought auxin signaling was extensively characterized in the last years, several pieces of the auxin-signaling puzzle remain to be assembled. Here we describe a novel regulator of auxin signal transduction, *JAGGED LATERAL ORGANS* (*JLO*), a member of the LATERAL ORGAN BOUNDARY DOMAIN transcription factor family. Our genetic studies and gene expression analyses implicate a role for JLO in the transcriptional regulation of the auxin receptors *TRANSPORT INHIBITOR RESPONSE 1* (*TIR1*) and *AUXIN SIGNALING F-BOX1* (*AFB1*). Reduced *TIR1/AFB1* expression in *jlo* mutants causes the stabilization of AUX/IAA proteins like BODENLOS (BDL). The resulting failure to release ARF transcription factors from repression is the cause for the misexpression of several auxin-regulated genes in *jlo* mutant background. These genes comprise e.g. members of the *PINFORMED* (*PIN*) and *PLETHORA* (*PLT*) families and *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*). Thus, *JLO* seems to be a central regulator of auxin signaling and distribution during *Arabidopsis* root development.