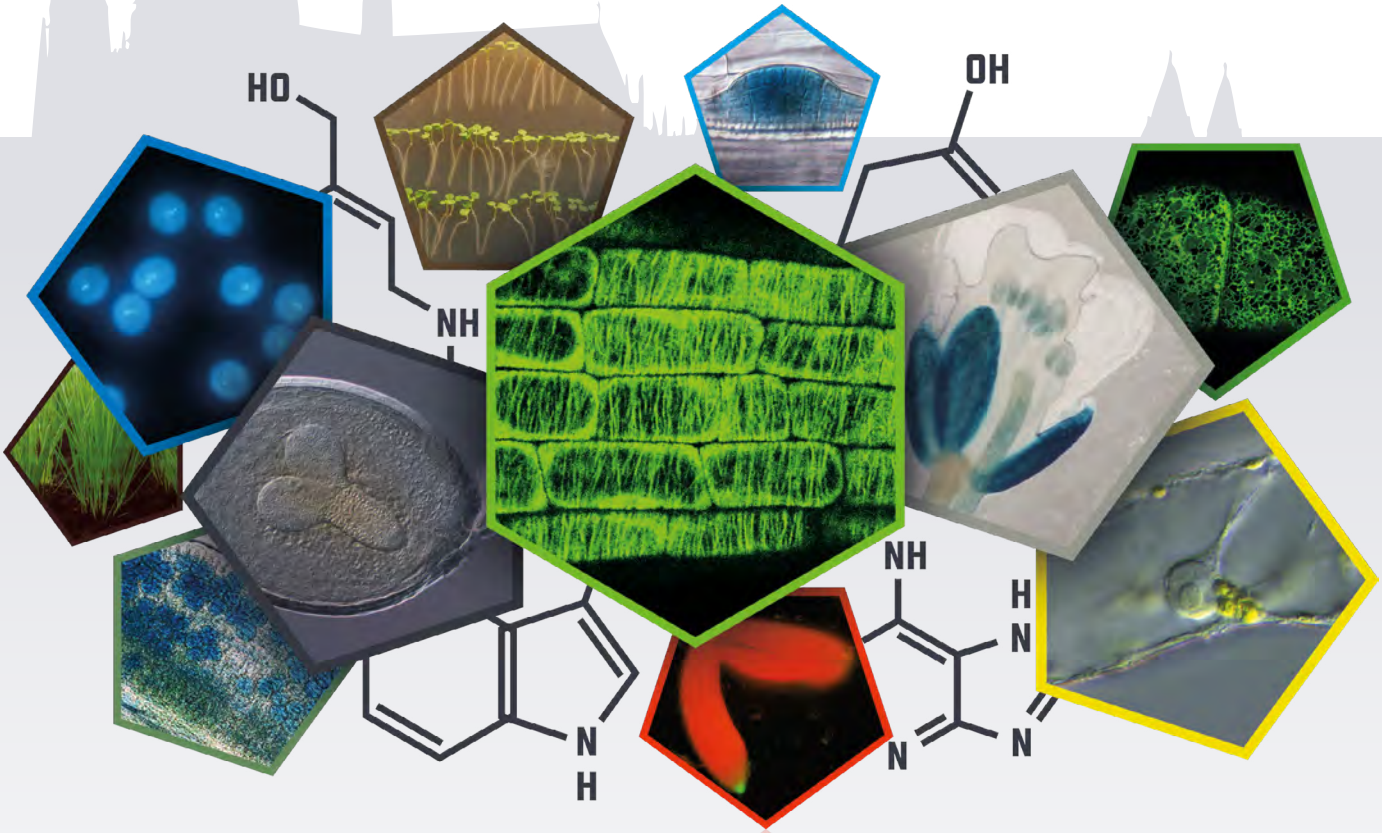
ACPD 2018

Auxins and Cytokinins in Plant Development

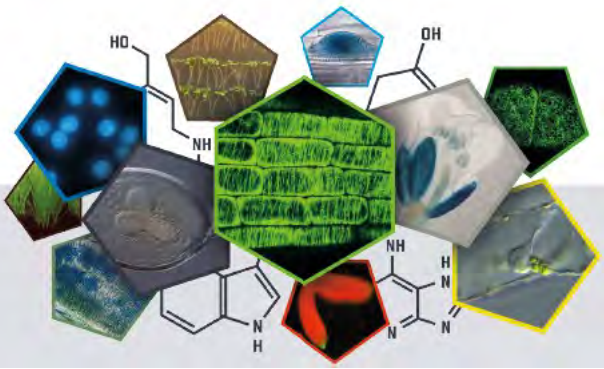
... and Interactions with Other Phytohormones

International Symposium 2018

July 1–5, 2018 | Prague, Czech Republic



BOOK OF ABSTRACTS



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ORAL PRESENTATIONS

O-01
Molecular networks orchestrating biomass productivity

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Plant organ growth is regulated by an exceedingly complex interplay of many genes and their interaction with the ever changing environment. The long-term goal of our research is to obtain a holistic understanding of plant organ growth. Numerous genes of which the modified expression enhances plant organ growth have now been identified, and a detailed study of these genes provided novel insights in the molecular machines driving growth. Plant hormones, including auxins and cytokinins, play a pivotal role in determining organ formation and size. I will review the various mechanisms orchestrating growth and demonstrate that profound knowledge on growth-enhancing genes offers excellent opportunities for yield enhancement of crops. Furthermore, evidence obtained both in the model plant Arabidopsis and in maize, demonstrated that the combination of multiple growth enhancing genes can have even more profound effects on organ sizes.

O-02
Auxin signaling and transport

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The plant hormone auxin is a versatile intercellular signal influencing virtually all aspect of plant life. It has a unique ability to be directionally transported within tissues forming local auxin maxima or gradients that are central to many developmental processes mediated by auxin. The key components of this polar auxin flow are plant-specific, auxin exporters from the PIN family showing typically polar, subcellular localization at the plasma membrane. PINs have been shown to mediate auxin export from cells in many homologous and heterologous systems but their structure and mechanism of their action are still unknown. In addition, the role(s) of some PIN proteins and other potential auxin transporters that were found at the endoplasmic reticulum remains mysterious.
On the other hand, auxin perception and signaling by the SCF^{TIR1} - AUX/IAA – ARF mechanism is well characterized from both functional and structural point of view. It has been believed that it is the major pathway, by which auxin modules gene transcription and thus plant development. This model applies very well to many processes including auxin-mediated growth promotion in shoot but is not applicable to another classical auxin effect – growth inhibition in root.
Here we will provide new insights into the mechanisms of auxin signaling mediating both transcriptional and non-transcriptional cellular responses. In addition, we present how different

auxin signaling pathways cooperatively regulate PIN-dependent auxin transport contributing to the self-organizing nature of plant development.

O-03

New Insights into Cytokinin Signaling

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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. These two-component signaling elements are partially functionally redundant in mediating the response to cytokinin and in various roles in regulating plant growth and development.
We are characterizing the mechanism underlying cytokinin signaling in both Arabidopsis and rice, and are exploring how this two-component signaling pathway modulates the many processes regulated by cytokinin. We have examined the cytokinin-regulated transcriptional network, including characterizing of the cytokinin-regulated transcriptome, binding of the type-B ARRs to their genomic targets, and the effects of cytokinin on chromatin accessibility across the genome. We have examined the role of downstream elements in cytokinin function, focusing on several cytokinin–regulated transcription factors. We have screened for novel elements in cytokinin signaling using a sensitized genetic background coupled with a new approach to identification of causative mutations in a parallel fashion. We have uncovered a link between cytokinin signaling and autophagy, focusing on the role of the type-A ARRs in this process. Finally, we have characterized the effect of disruption of cytokinin signaling elements in the monocot rice.

O-04

"Keeping the balance" – mechanisms controlling auxin and cytokinin homeostasis and their regulation

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Auxin (IAA) and cytokinins (CKs) are major regulators of plant growth and development, and their non-uniform distribution between cells and tissues underlie spatiotemporal coordination of many developmental events and responses to environmental stimuli.
Control of IAA gradients and the formation of IAA maxima/minima involve regulation of both metabolic and transport processes. We have shown that 2-oxindole-3-acetic acid (oxIAA) is the major primary IAA catabolite in *Arabidopsis thaliana* tissues, formed by the highly root-expressed, cytoplasmically localized IAA oxidase DIOXYGENASE FOR AUXIN OXIDATION 1 (DAO1). We also showed that IAA conjugation and catabolism regulate auxin levels in Arabidopsis in a highly redundant manner, in order to maintain auxin concentrations at optimal levels for plant growth and development. Similar processes regulate IAA levels also in other plant species.
Both IAA and CKs have been shown to form concentration gradients in the root, with the highest concentrations observed in distinct cell types of the root apex. Recent data suggest that some genes involved in IAA biosynthesis are under transcriptional control by specific type B response regulators (ARRs), showing that CKs and IAA interact also at the metabolic level. These and other data suggest that CKs and IAA can regulate each other's biosynthesis and degradation, a potentially important mechanism for fine-tuning plant hormone levels, e.g. in developing lateral root primordia and in the root apex.

O-05
Auxin and cytokinin homeostasis on cellular and subcellular levels

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Auxins and cytokinins (CKs) play crucial roles in the control of various physiological and developmental processes. In our previous research, we have made significant efforts in clarifying the regulation of auxin and CK metabolic pathways, including biosynthesis, conjugation and degradation, and further demonstrating how these pathways contribute to auxin and CK homeostasis. However, our knowledge about the distribution and compartmentation of phytohormones and their metabolites at the cellular level is still very limited.
We focus on efficient cell and organelle isolation, combining different approaches such as density gradient ultracentrifugation or fluorescence-assisted cell/organelle sorting (FACS/FAOS) with micropurification protocols and ultra-sensitive mass spectrometry techniques (LC-MS). New analytical tools will provide comprehensive insights into plant hormone regulatory networks, such as detailed distribution of plant hormones in specific tissues, cells and organelles. This will allow us to obtain e.g. nuclear fractions of high-purity and intactness in an adequate yield suitable for future analytical assessments. Our preliminary data point out to the fact that auxin and CK profiles in plant cell are quite complex and do not include just expected active molecules, but also other key representatives that covers phytohormone biosynthesis, conjugation and degradation. By employing these novel methods, we will be able to gain a much better understanding of how genetic and experimental manipulations affect plant hormone levels, which will foster a more complete understanding of how these hormones act.

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O-06

New generation of urea-derived inhibitors of cytokinin oxidase/dehydrogenase for future *in vivo* studies

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Homeostasis of cytokinins is regulated by a flavoenzyme family of cytokinin oxidases/dehydrogenases (CKO/CKX) that irreversibly oxidize these plant hormones. Thidiazuron (N-phenyl- N′-1,2,3-thiadiazol-5-yl urea, TDZ) and N-(2-chloro-pyridin-4-yl)-N´-phenylurea (CPPU) are well-known CKX inhibitors, which were used in many previous studies. These compounds compete with substrates for the binding site above the isoalloxazine plane of FAD cofactor of CKX. In general, CKX inhibitors might increase the lifetime of endogenous cytokinins and affect different cytokinin functions, thereby having positive effects on seed filling, delayed senescence and stress tolerance toward biotic and abiotic stresses and thus improving crop yield. Development of potent CKX inhibitors is meaningful with respect to the ban on genetically engineered food and crops in EU. Up to date, we designed more than 50 new phenylureas derived from TDZ, CPPU and others with various substitutions on their aromatic rings. We used several CKX isoforms from maize (*Zea mays*) including ZmCKX1, ZmCKX2, ZmCKX4a, ZmCKX5 and ZmCKX8 to study the inhibitory strength of new inhibitors by analyzing enzyme kinetics as well as their binding mode by X-ray crystallography. We identified several compounds with IC_{50} values in nanomolar range and solved crystal structure complexes up to 1.9 Å resolution. Urea derivatives are also well known to strongly activate the cytokinin receptors and trigger the cytokinin signaling. As highly specific CKX inhibitors without undesired side effects are of major interest, the new compounds are currently tested for cytokinin activity in several cytokinin bioassays and receptor binding assays to identify attractive candidates for *in planta* trials.

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O-07

Contribution of DAO-mediated IAA inactivation to auxin homestasis in Norway spruce seedlings

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Plants finely control the levels and concentration gradients of the hormone auxin to coordinate and modulate plant growth and development. This can be achieved by regulating metabolism and homeostasis of the major auxin form, indole-3-acetic acid (IAA), through its anabolic and catabolic pathways. The two main routes of IAA inactivation are the conjugation of IAA to amino acids and sugars, *e.g.*, glutamate (IAGlu) and aspartate (IAAsp) and the oxidation of IAA to 2-oxindole-3-acetic acid (oxIAA). OxIAA is the primary IAA catabolic product in monocots and dicots. Its formation has been recently ascribed to the activity of 2-oxoglutarate-dependent-Fe(II) dioxygenases (2OGDs), DIOXYGENASE FOR AUXIN OXIDATION (DAO), whose members have only been identified in apple, rice and *Arabidopsis* to date. Here, we show that oxIAA is a native metabolite also in seedlings of the gymnosperm *Picea abies* (Norway spruce), although the conjugation to amino acids seems to be the main inactivation process compared to *Arabidopsis*. To understand the contribution of the oxIAA pathway to the IAA catabolism, we have identified and cloned putative spruce homologs to the *ArabidopsisDAOs*. We are currently testing their ability to catalyze the oxidation of IAA into oxIAA by conducting *in vitro* enzyme assays and by generating target-specific knockout spruce plantlets using CRISPR/Cas9 RNPs.

O-08

Biosynthesis and transport of cytokinin variations control the specificity of the action in shoot growth and development

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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. We have demonstrated that *IPTs*, *CYP735As*, and *LOGs*, which are key genes for *de novo* CK biosynthesis, are expressed in various parts during growth and development, and differentially regulate the synthesis of N^6-(Δ^2-isopentenyl)adenine (iP) and *trans*-zeatin (tZ). In addition, *ABCG14*, a member of ABC transporter family, has been recently identified as a key gene for root-to-shoot translocation of CKs via xylem. 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. This regulation is one of the qualitative controls of CK action involved in shoot growth regulation by root-borne signal. Furthermore, our recent studies indicate that physiological effects of root-derived tZ via xylem on shoot morphology were different from those of tZ riboside. These findings suggest that complex action of long-distantly transported cytokinins could be organized by the side chain structure and the dependency of the activation pathway. The dual long-distance cytokinin signaling system would be important for fine-tuning shoot growth manner in response to environmental conditions. We will outline our recent progress in CK study, and discuss the physiological significance of regulation of CK action to optimize growth and development at whole plant level.

O-09

Characterisation of immediate-response cytokinin metabolism in Arabidopsis: differences in pathway kinetics determine the natural spectrum of cytokinin metabolites

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The breadth of cytokinin metabolic spectrum in plants suggests cytokinin metabolism to be the dominant regulatory mechanism of cytokinin function. Kinetics of several enzymes involved in cytokinin metabolism have previously been investigated *in vitro*, however, little is still known about their activity, directionality and kinetics *in planta*.
In our study 14-day-old Arabidopsis seedlings were treated with selected cytokinins in a short time scale (up to 120 min) and resulting cytokinin levels in roots and shoots were measured by HPLC-MS. After an interactive visualisation, the data were evaluated qualitatively and through computational modelling. Optimization of the mathematical models provided estimates of the kinetic rates of major reactions. Subsequently, a sensitivity analysis and statistical evaluation of the optimisation results helped determine the reliability of the parameter estimates as well as test several hypotheses concerning the conversion of iP-type to tZ-type cytokinins.

Except for increased levels of tZ metabolites after application of iP, we observed no conversions between the distinct cytokinin types (iP, tZ, DHZ, cZ). In particular, no evidence of isomerization between the tZ and cZ types was observed. Also, no elevation of DHZ-type cytokinins was found after two-hour application of tZ. This raises the question how, when and in what amounts DHZ is naturally produced. Next, after application of the bases of all four cytokinin types, low levels of ribosides were observed in contrast to moderately abundant riboside phosphates, suggesting dominance of APT pathway over a stepwise conversion of bases to ribosides and then to riboside phosphates. Concerning N-glucosides, iP N-glucosides were not converted back to iP. Contrastingly, tZ N-glucosides were metabolised to tZ bases, thus affecting the whole metabolic spectrum, in which the four cytokinin types differed in their ratio of riboside phosphates (dominant in tZ) and N-glucosides (dominant in iP).

O-10

So does cytokinin inhibit or promote root growth? The *ipt29*-short-root story

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Cytokinins' (CKs') inhibitory effect on root growth was one of the early discoveries, establishing the hormonal class as indispensable for regulation of plant development. Unlike most phytohormone classes, CKs are represented by more than one active molecule. Multiple mutant lines, blocking specific parts of CK biosynthetic pathways, have enabled research in plants with deficiencies in specific CK-types. While most of these mutants have confirmed the impeding effect of the hormone on root growth, the *ipt29* double mutant, surprisingly, has reduced primary root length compared to wild type. This mutant is impaired in *cis*-zeatin (*c*Z) production, a CK-type that had been considered inactive in the past.

Here we have further investigated the intriguing *ipt29* root phenotype, opposite to CK known functions, and the (bio)activity of *c*Z on site. Our data suggest that despite the *ipt29* short-root phenotype, cZ application has a negative impact on primary root growth and can activate CK

response in the stele, where it is perceived by CK-receptor homodimers. Additional grafting experiments revealed that the root phenotype of *ipt29* depends pivotally on local signaling which did not relate to CKs. Latest data showed that *ipt29* has reduced auxin levels, suggesting that IPT2 and/or IPT9 could affect auxin metabolism and thus root growth.

O-11

The biosynthesis and signalling of cytokinins during the formation of tumours in the *Ustilago maydis-Zea mays* pathosystem

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Tumour formation is a characteristic symptom of the common smut of corn disease caused by the fungus *Ustilago maydis*. During the development of tumours, cytokinin levels are elevated. Cytokinins are plant hormones that stimulate cell growth and cell division. Although the level of cytokinins are increased, the biosynthetic origins of these cytokinins have not been determined - Are they produced by the corn or the fungus? Both can produce cytokinins when grown independently. In addition, it is unknown as to whether the increased production of cytokinins contribute towards the formation of tumours. To investigate these phenomena, several genes and proteins potentially involved in the biosynthesis and signalling of cytokinins were identified in both organisms. The plant proteins responsible the biosynthesis and signalling of cytokinins are different from the fungal proteins and this allowed us to determine the changes in protein transcript levels over the course of pathogenesis by using reverse transcription PCR. The results indicate that the increase in cytokinins originate from both the corn and the fungus proteins. In addition, increased cytokinin signalling was also observed during the formation of tumours. This led a model of the interaction in which the fungus secretes active cytokinins into the corn host. The increased production of active cytokinins leads to increased cytokinin signalling which promotes excessive cell division and the formation of tumours. The enhanced insight into how *U. maydis* induces the formation of tumours will aid in developing molecular approaches to inhibit tumour formation and mitigate the negative impacts of fungal diseases.

O-12

Brassinosteroids regulate glucosinolate biosynthesis in *Arabidopsis thaliana*

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Plants must constantly adjust their growth and defense responses to deal with the wide variety of stresses they encounter in their environment. Among phytohormones, brassinosteroids (BRs) are an important group of plant steroid hormones involved in numerous aspects of the plant lifecycle including growth, development, and responses to various stresses including

insects. Here, we show that BRs regulate glucosinolate (GS) biosynthesis and function in insect herbivory. Preference tests and larval feeding experiments using the generalist herbivore, diamondback moth (*Plutella xylostella*), revealed that the larvae of this moth prefer to feed on *Arabidopsis thaliana brassinosteroid insensitive 1* (*bri1-5*) plants than on wild-type Ws-2 or BRI1-Flag (*bri1-5* background) transgenic plants, which leads an increase in larval weight. Analysis of GS contents showed that 3-(methylsulfinyl) propyl glucosinolate (C3) levels were higher in *bri1-5* than in Ws2 and BRI1-Flag transgenic plants, whereas sinigrin (2-propenylglucosinolate), glucoerucin (4-methylthiobutylglucosinolate), and glucobrassicin (indol-3-ylmethylglucosinolate) levels were lower in this mutant. We investigated the effect of brassinolide (BL) on GS biosynthesis in Arabidopsis and radish (*Raphanus sativus* L.) by monitoring the expression levels of GS biosynthetic genes, including *MAM1, MAM3, BCAT4*, and *AOP2*, which increased in a BL-dependent manner. These results suggest that BRs regulate GS profiles in higher plants, which function in defense responses against insects.

O-13
Auxin and drought response in Arabidopsis

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Auxin controls growth and diverse physiological processes through a complex transcriptional network that includes thousands of genes. Auxin regulates gene expression by promoting the degradation of transcriptional repressors called Aux/IAA proteins. The 29 *Aux/IAA* genes in *Arabidopsis* exhibit unique but partially overlapping patterns of expression. Although some studies have suggested that individual *Aux/IAA* genes have specialized function, genetic analyses of the family have been limited by the lack of loss-of-function phenotypes, presumably because of overlapping function. Further, with a few exceptions, our knowledge of the factors that regulate *Aux/IAA* expression is limited. We hypothesize that transcriptional control of *Aux/IAA* genes plays a central role in the establishment of the auxin-signaling pathways that regulate organogenesis, growth, and environmental response. To identify transcription factors that regulate the *Aux/IAA* genes, we performed a yeast-1-hybrid screen with 15 *Aux/IAA* promoters against ~2000 Arabidopsis TFs. Our results indicate that the *Aux/IAA* genes are regulated by many transcription factors implicated in diverse processes. We have focused on regulation of *Aux/IAA* by the DREB2A/B transcription factors. The DREB2 proteins have been described as master regulators of ABA-independent responses to drought, heat and cold. Consistent with this, our genetic studies indicate that several *Aux/IAA* genes are required for drought tolerance. In my talk I will describe results that explain this requirement.

O-14
Mapping auxin receptor selectivity in three dimensions and over evolution

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Auxin perception is dominated by a small family of receptors related to Transport Inhibitor Response1 (TIR1), and is initiated by the hormone indole-3-acetic acid in dialogue with a deep binding pocket. The receptors show small differences in binding affinity for different auxin scaffolds and structure-activity mapping of these preferences describes in three dimensions and at atomic resolution the permitted ligands in each case. We have used the full range of auxin herbicide scaffolds, TIR1 and AFB5 as representative members of the receptor clades in Arabidopsis, and the TIR1 homologues from the moss *Physcomitrella patens* and liverwort *Marchantia polymorpha*. Molecular field point analysis of the data transforms the structure-activity data into pharmacophoric atlases and allows us to plot the basis of ancestrally-conserved selectivity in TIR1. These tools provide great resolution for our studies of the basis of auxin recognition and may help identify novel auxins.

O-15

Root growth inhibition by auxin – an old story full of surprises

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The phytohormone auxin is the information carrier in many developmental and physiological instances in the plant body. This is prominent during the gravitropic response of the root: the gravity stimulus is sensed in the columella cells of the root tip, which leads to auxin transport to the lower epidermis of the root, where auxin triggers a rapid growth inhibition response. The canonical auxin receptor TIR1/ABF-Aux/IAA acts through regulation of gene transcription, but many cellular reactions to auxin are too rapid to be explicable by this mechanism. Here we combined live cell imaging with microfluidics and computational modeling to reanalyze this classical case in plant biology – the growth reaction of Arabidopsis roots to auxin. We show that roots react to addition and removal of auxin by almost instantaneous adaptation of growth rate. Mutant analysis and modelling of auxin fluxes demonstrated that this reaction depends on intracellular level of auxin. While the process does not involve TIR1-dependent transcriptional reprogramming, the signaling apparently starts at and requires the TIR1/AFB-Aux/IAA coreceptor, and the root growth rate is negatively correlated with the formation of the receptor

complex, hinting to a novel branch of this signaling pathway. Our results challenge the current understanding of the root growth regulation by auxin as well as the mechanism of the canonical auxin coreceptor pathway.

O-16

Auxin receptors for rapid protoplast swelling and rapid growth responses – a critical comparison

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From the beginning ABP1 seemed to be a good candidate auxin receptor for responses too rapid to involve transcriptional regulation, including rapid auxin-induced growth. This view was supported by research on certain electrophysiological auxin effects and on auxin dependent protoplast swelling, both of which were affected by ABP1-related immunological tools. Since vital *abp1* mutants have been finally created recently, we now have the tools at hands to test some of these earlier claims and ideas.
We could demonstrate that auxin dependent protoplast swelling was abolished in *abp1*-C1 and *abp1*-TD1, proving that ABP1 is needed for the swelling response. We found that swelling was also induced by other auxin analogs including PEO-IAA, but not by 5-F-IAA. C-terminal peptides, which are thought to bind to a docking protein complex at the plasma membrane, trigger auxin independent swelling in wild type and mutant protoplasts.
To check if these rapid effects on cell volume relate to elongation growth at the organ level, we developed imaging-based auxanometers for recording rapid growth responses in excised hypocotyls and intact roots of *Arabidopsis* seedlings. The results were not in line with the idea that ABP1 is needed for rapid elongation growth, as the mutants displayed wild-type- like auxin dependent growth. Also 5F-IAA was nearly as effective as IAA, while PEO-IAA did not induce any growth. This pattern of auxin specificity is very different from what we saw in ABP1-dependent protoplast swelling, but the same that emerges in TIR/AFB dependent auxin-induced gene expression.
We also investigated the auxin specificity in a number of monocot species including maize, and used the maize coleoptile system to check a number of other auxin effects suggested to be causally related to rapid cell elongation such as H^+-secretion. Evidently growth is regulated by a receptor other than ABP1, and we will discuss the possibility of TIR/AFB being this receptor.

O-17

Molecular dynamics determination of the auxin binding pathway on TIR1

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Both natural and synthetic auxins bind to the TIR1 receptor, whose active site is at the bottom of a deep pocket. The binding process displays a high degree of selectivity, which has been suggested to be due to the sequence of interactions of potential ligands with the structural features of TIR1 they meet as they descend the pocket. We analyze the binding mechanism of IAA with molecular dynamics simulations, including enhanced sampling methods such as low-mode search and nudged elastic band calculations. Our results show that TIR1 undergoes a ligand-induced structural change that opens the otherwise closed pocket, allowing the binding of IAA. In addition, we identify an actual physical pathway for the descent to the binding site, whose driver is the interaction with several residues, mainly Arg-489 and Arg-403, which transport IAA by electrostatically locking it onto them in sequence. In turn, the binding stabilizes the conformation of the pocket, which would be energetically prohibited in the absence of the ligand, making it available for degron binding.

O-18

The many facets of cytokinin as a signal regulating plant development and stress responses

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The core cytokinin signaling pathway has been established more than a decade ago. Recently, further details like the cellular topology of the signaling pathway have added to a better understanding. New tools like constitutive active cytokinin receptor variants named *repressor of cytokinin deficiency2/3* (*rock2/3*) enable novel approaches to alter cytokinin homeostasis in a cell-autonomous fashion. One of the great challenges is to understand how the numerous and diverse activities of cytokinin are realized. There are several levels of signal specification including subcellular localization, metabolic conversions and transport, specificity of receptor signal recognition, signal transfer and coupling to downstream processes, mostly resulting in modulating the output of the transcriptional machinery. This is principally achieved by B-type response regulators, which may act alone or in combination with other transcription factor families. Crosstalk with other signalling pathways adds an additional level of complexity and allows for fine-tuning of cytokinin action. Selected examples of specific cytokinin signaling pathways will be presented. My lab is currently interested to investigate a number of understudied or novel activities of the hormone in development, such as regulation of seed germination, the juvenile-to-adult transition of leaves and flowering time. Cytokinin regulates also a novel type of abiotic stress named altered photoperiod stress. Other work addresses the question whether our knowhow on modulating cytokinin signaling can be used to increase the performance of crop plants, which is attempted in oilseed rape, barley and poplar.

O-19

BIL1-mediated phosphorylation of ARF5 integrates TDIF/TDR and cytokinin signaling into vascular cambial activity

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Vascular cambium development in plants is crucial to generate vascular tissues and mechanical strength. Phytohormones and mobile peptides are key regulators of vascular cambial activity; however, their coordinated regulatory mechanisms are largely unknown. Here, we show that BIN2-like 1 (BIL1), a glycogen synthase kinase-3 (GSK3), suppresses the cytokinin response via phosphorylation of ARF5 to regulate cambial activity. In turn, the tracheary element differentiation inhibitory factor (TDIF)/TDIF RECEPTOR (TDIF/TDR) module functions to constitutively recruit BIL1 that dissociates from ARF5, thereby attenuating ARF5 transcriptional activity. ARF5 targets *ARR7* (*ARABIDOPSIS RESPONSE REGULATOR7*), a negative regulator of cytokinin signaling, to suppress cambial activity. These combined results suggest that BIL1 is a key mediator linking peptide signaling with the auxin/cytokinin network in the maintenance of cambial activity.

O-20

Structural insights into the specificity of multistep phosphorelay signaling in Eukaryotes

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The multistep phosphorelay (MSP) is a backbone signaling pathway mediating responses to a plethora of stimuli in bacteria, fungi, algae and plants. However, the mechanisms allowing integration of various signals, while maintaining the specificity of individual inputs in the MSP signaling of Eukaryotes remained elusive. Here, for the first time in the eukaryotic system, we describe structural determinants of signaling specificity in the *Arabidopsis thaliana* MSP. We determined the structure of AHP2 acting downstream of sensor kinase CKI1. Molecular dynamics simulations complemented by NMR measurements identified residues responsible for the recognition of AHP2 by the receiver domain of CKI1 ($CKI1_{RD}$). Strong electrostatic interactions result in the repositioning of both partners and formation of distinct interaction network when compared with the related AHP1-$AHK5_{RD}$ complex. Using the knowledge, we rewired AHP1 and made it interact with its non-cognate partner $CKI1_{RD}$. Our data show that the relative orientation of the interaction partners, controlled by a small number of AHP residues, underlies molecular recognition in CKI1 signaling, providing evidence for a unique mechanism determining MSP specificity in Eukaryotes.
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O-21
Cytokinin perception in potato: receptor properties and expression

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Potato is the most economically important non-cereal food crop. Tuber formation in potato is regulated by phytohormones, cytokinins in particular. The present work was aimed to study

cytokinin signal perception in potato. The sequenced potato genome of doubled monoploid Phureja was used for bioinformatic analysis and as a tool for identification of cytokinin receptors from autotetraploid potato cv. Désirée. All basic elements of multistep phosphorelay (MSP) required for cytokinin signal transduction were identified in Phureja genome, including three genes orthologous to three cytokinin receptor genes (*AHK 2-4*) of Arabidopsis. As distinct from Phureja, autotetraploid potato contains at least two allelic isoforms of each receptor type. Putative receptor genes from Désirée plants were cloned, sequenced and expressed, and main characteristics of encoded proteins, firstly their consensus motifs, structure models, ligand-binding properties, and the ability to transmit cytokinin signal, were determined. In all studied aspects the predicted sensor histidine kinases met the requirements for genuine cytokinin receptors. All receptors bind cytokinin stronger in basic (pH 7–9) than acidic (pH 5–7) pH range. This indicates the intracellular functioning of potato cytokinin receptors. Expression of potato cytokinin receptors was found to be organ-specific and sensitive to growth conditions, particularly to sucrose content. Our results provide a solid basis for further in-depth study of cytokinin signaling system and biotechnological improvement of potato.
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O-22

Cellulose biosynthesis inhibition reduces cell cycle activity in a nitrate reductase- and cytokinin-dependent manner

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Plant cell wall metabolism must adapt to developmental processes like cell elongation and division. This is exemplified by the tightly regulated cellulose deposition during cytokinesis when a new cell wall must be generated to separate the two daughter cells forming. However, processes like the cell cycle are dependent on the functional integrity of the cell wall to allow successful cell cycle progression and completion. This is illustrated by results

from *Saccharomyces cerevisiae* where the cell wall integrity (CWI) maintenance mechanism influences cell cycle regulation progression.
Here we investigated in *Arabidopsis thaliana* seedlings both how the cell cycle responds to cell wall damage (CWD) impairing CWI and the mode of action of the regulatory mechanism responsible. We found that CWD generated by cellulose biosynthesis inhibition leads to osmo-sensitive inhibition of root growth and cell cycle progression. Genetic analysis showed that none of the known CWI signaling components are required, instead intact NIA1 NIA2, encoding nitrate reductases implicated in NO metabolism, are essential. A phenotypic characterization showed that co-treatments with zeatin could rescue the observed inhibition in a concentration dependent manner. Quantification of cytokinin levels detected CWD-induced, osmo-sensitive changes in certain cytokinins. These results were followed up with additional genetic and gene expression profiling studies suggesting that CWD-induced cytokinin degradation is responsible for the observed effects on cell cycle progression. The available data suggest a NIA1 NIA2 dependent process may be responsible for changes in the levels of certain cytokinins, which in turn seem to regulate cell cycle activity.

O-23
Origin and evolution of the nuclear auxin response system

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The small signaling molecule auxin controls numerous developmental processes in land plants, acting mostly by regulating gene expression. Auxin response proteins are represented by large families of diverse functions, but neither their origin nor the evolution of diversity is understood. We have used a deep phylogenomics approach to reconstruct both the origin and the evolutionary trajectory of all nuclear auxin response protein families. We found that, while all subdomains found in auxin response proteins are ancient, a complete auxin response mechanism is limited to land plants. Functional phylogenomics predicts defined steps in the evolution of response system properties. We have performed a comparative transcriptome analysis across six ancient lineages of charophytic green algae, bryophytes and ferns and show how these innovations have shaped a sophisticated response mechanism. We discovered the existence of a mechanistically independent transcriptional auxin response system in green algae. Finally, genetic analysis in the liverwort Marchantia polymorpha revealed unexpected contributions of ancient non-canonical proteins in auxin response as well as auxin-unrelated function of core transcription factors. Our study provides a functional evolutionary framework for understanding diverse functions of the auxin signal. I will present our progress in dissecting the evolutionary, structural and functional basis of the mechanism mediating auxin-dependent gene regulation.

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O-24

Auxin response factor (ARF) activators are transcriptionally regulated by gene-specific repressor network

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Auxin regulates plant growth and development through the transcription factors of the AUXIN RESPONSE FACTOR (ARF) gene family. Most notably in *Arabidopsis thaliana* ARF5, 6, 7, 8 and 19 activate expression of target genes in response to auxin. These five ARF activators control both variable and overlapping processes during plant development including regulation of growth at the root and the shoot apical meristems, lateral root and axillary shoot formation. Each of the five ARF activators shows unique tissue-specific expression patterns in the root and the shoot associated with their distinct functions. This tissue-specific expression is likely derived from the differences in the control of ARF activator transcription. In this study the upstream regulators of ARF5, 6, 7, 8 and 19 transcription were identified. This was achieved by utilizing a high-throughput yeast one-hybrid (Y1H) method. Transient protoplast assays indicate that each ARF activator is controlled by a set of specific transcriptional regulators and that the majority of these regulators are repressors of ARF transcription *in planta*. Mutants of the regulatory transcription factors were utilized to additionally investigate their function *in planta*. These mutants display auxin-related developmental phenotypes in the root and the shoot including alterations in growth kinetics, emergence of lateral organs, responses to auxin as well as altered expression of ARF activators. This study provides clues to how differential expression of ARFs contributes to specificity in auxin responses.

O-25

Cytokinin response regulators are indispensable for organ formation in *Marchantia polymorpha*

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Cytokinins play an essential role in plant growth and development. Type-B response regulators (RRBs) are transcription factors that control cytokinin-responsive genes downstream of the two-component signaling pathway. Type-A response regulators (RRAs), one of the targets of RRBs, repress the cytokinin signaling, thereby alleviating the response. Genetic analyses using *Arabidopsis* mutants showed that both types of RRs are involved in various developmental processes; however, how RRs coordinate many downstream events is largely unknown. To identify fundamental mechanisms underlying RR-mediated control of cytokinin response, we are using the basal land plant, *Marchantia polymorpha*. Our phylogenetic analysis showed that *M. polymorpha* has only one gene for each type-A and -B RRs, termed Mp*RRA* and Mp*RRB*, respectively. Mp*RRA* was transcriptionally up-regulated by Mp*RRB*, and Mp*RRA*-overexpressing lines displayed the phenotype with low levels of cytokinin signaling, suggesting a conserved negative feedback attenuating the cytokinin signaling. The transgenic lines defective in cytokinin signaling, such as Mp*rrb* knockout line, formed less or no gemma cup and more rhizoids, indicating that cytokinins are involved in the formation of new vegetative organs at the apical notch. We shall discuss how Mp*RRB* regulates the downstream events based on the mutant phenotype and transcriptome analysis.

O-26

Identification of a cytokinin-signalling Type-B Response Regulator (RRB) transcription factor regulating two symbiotic nodulation genes in *Medicago truncatula*

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Formation of nitrogen-fixing nodules on legume roots requires the infection by soil bacteria collectively referred to as Rhizobia, and the initiation of cell divisions in the root cortex, several cell layers away from the infection site in the root epidermis. Cytokinins, a class of plant hormones, play a major role in symbiotic nodule organogenesis since they are critical for initiating root cortical cell divisions at the onset of nodule initiation. Cytokinin signalling leads to the activation of Type-B Response Regulators (RRBs) which are transcription factors regulating the expression of cytokinin primary response genes. *Nodulation Signalling Pathway 2* (*NSP2*) and *Cell Cycle Switch 52A* (*CCS52A*) are two genes involved in nodule organogenesis which expression is regulated by cytokinins, indicating that they are therefore direct or indirect targets of RRBs. Amongst the 10 RRBs expressed in *Medicago truncatula* nodules, MtRRB3 appeared based on a protoplast assay as the best candidate to regulate the expression of these two genes, consistently with ChIP-qPCR assays showing that RRB3 interacts with *NSP2* and *CCS52A* promoters. Importantly, the expression pattern of a translational RRB3-GUS fusion overlaps with the *pNSP2-GUS* and *pCCS52A-GUS* transcriptional fusions in roots and nodules. To examine the role of RRB3 in nodulation, plants silenced for MtRRB3 by RNAi and *rrb3* mutant plants were generated, which both showed a significant decrease in nodule number. Further investigations are in progress to decipher the regulation of *NSP2* and *CCS52A* by RRB3 *in planta* in the context of symbiotic nodulation.

O-27
The role of Cytokinin signaling during Vascular proliferation

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To form three-dimensional structures, plants change the orientation of their cell divisions to enable both radial and longitudinal growth. A heterodimer formed by the bHLH transcription factors TARGET OF MONOPTEROS5 (TMO5) and LONESOME HIGHWAY (LHW) specifically triggers periclinal cell divisions that contribute to vascular proliferation by promoting local biosynthesis of cytokinin (CK). However, how CK is involved in the control of these specific divisions is largely unknown. To unravel the specific CK signaling components involved in this process, we analyzed which of the downstream ARABIDOPSIS RESPONSE REGULATORS (ARR) transcription factors in the CK signaling pathway could be involved in this developmental process. Transcriptional reporter lines showed ARR expression in meristematic vascular cells for more members then the previously described ARR1, 10 and 12. This suggests a role for these other ARRs during vascular development. Moreover, a specific subset of these showed ectopic expression outside of their expression domain upon TMO5/LHW induction, but not after

exogenous CK treatments. Thus, besides CK biosynthesis, CK signaling could also be a downstream target of the TMO5/LHW complex.

O-28
Epigenetic compensation of a genetic lesion

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Organisms have evolved strategies to cope with external and internal disturbances, allowing them to maintain form and function despite a changing environment or genetic variations. Genetic compensation alleviates the phenotypic consequences of potentially harmful mutations, and manifests as adaptive changes in the transcription profile. Recently, it was described in different organisms that conditional gene knockdowns are phenotypically more detrimental compared to permanent mutations of the same genes. There are two possible explanations: strong phenotypes caused by transient knock-down approaches are due to toxicity or off-target effects, or the weak phenotypes of the permanent mutants are the result of genetic compensation. I will report that epigenetic modifications are involved in compensatory transcriptional induction of the cytokinin-degrading enzyme CYTOKININ OXIDASE 2 (CKX2) in *Arabidopsis thaliana*, to ameliorate ectopic signaling in the embryo root meristem caused by lesions in the ARABIDOPSIS RESPONSE REGULATOR 7 and ARR15 genes, while the induced RPS5A>ALC>ARR7(RNAi), *arr15* mutant fails to trigger the compensation.

O-29
Genetic and hormonal control of vascular cell proliferation

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The plant vascular system develops from a handful of provascular initial cells in the early embryo into a whole range of different cell types in the mature plant. In order to account for such proliferation and to generate this kind of diversity, vascular tissue development relies on a

large number of highly oriented cell divisions. Control of these divisions occurs in part through the TARGET OF MONOPTEROS 5/ LONESONE HIGHWAY (TMO5/LHW) dimers of bHLH transcription factors and their homologs. The cytokinin (CK) biosynthetic gene *LONELY GUY4* (*LOG4*) and its close homolog *LOG3* were identified as direct targets of the TMO5/LHW dimer complex, indicating that CK biosynthesis plays a crucial role in this developmental process. In order to understand how the TMO5/LHW-dependent CK biosynthesis controls vascular cell proliferation, we performed a detailed transcriptomics experiment. This dataset allowed us to identify consecutive waves of transcriptional activation and subsequent network analysis pinpointed several transcription factors as main hubs in this inferred network for TMO5/LHW-dependent induction of cell divisions. Here, I will highlight our current progress in understanding how these novel TFs control specific TMO5/LHW downstream responses leading to oriented cell divisions during vascular development.

O-30

WUSCHEL provides robustness to apical stem cell fate by pathway wide control of auxin signaling

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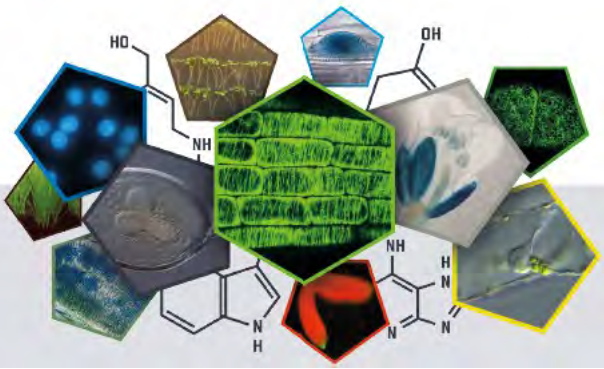
During plant development and growth, dynamic phytohormone signals need to be translated into spatially precise and temporally stable gene expression states, which in turn define cell fate. In the context of the shoot apical meristem, local accumulation of auxin at the periphery triggers organ initiation, while at the same time, centrally located stem cells are continuously maintained despite abundant auxin signaling input. We have found that spatial specificity in the auxin response is achieved by direct, pathway-wide transcriptional control by the WUSCHEL transcription factor in stem cells. Adding another layer of complexity, auxin signaling output is required for stem cell maintenance, suggesting that gating auxin response is one of the central activities of WUSCHEL. Our results reveal the mechanisms of a complex regulatory system that confers robustness against fluctuations of a mobile and highly potent developmental signal and thus prevents termination of an essential stem cell pool.



O-31

A molecular rheostat adjusts auxin flux to promote root protophloem differentiation

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Auxin impinges upon plant development through its distinct concentration-dependent effects. In the *Arabidopsis thaliana* (Arabidopsis) root tip, polar auxin transport by PIN-FORMED (PIN) proteins creates a local auxin accumulation that is required for stem cell niche maintenance. Proximally, stem cell daughters divide repeatedly before they eventually differentiate. This developmental gradient is accompanied by a gradual decrease in auxin as cells divide, followed by a gradual increase as they differentiate. However, the timing of differentiation is not uniform across cell files. For instance, developing protophloem sieve elements (PPSEs), which are essential for root meristem maintenance, differentiate while neighboring cell files still divide. Here we provide evidence that PPSE differentiation involves local steepening of the post-meristematic auxin gradient. BREVIS RADIX (BRX) and the AGC family PROTEIN KINASE ASSOCIATED WITH BRX (PAX) are plasma membrane-associated, polarly localized proteins that co-localize with PINs at the rootward end of developing PPSEs. Both, *brx* and *pax* loss-of-function mutants display impaired PPSE differentiation. Similar to other AGC family protein kinases, PAX activates PIN-mediated auxin transport, but BRX strongly dampens this stimulation of auxin efflux. While efficient BRX plasma membrane localization depends on PAX, auxin negatively regulates BRX plasma membrane association and promotes PAX activity. Thus, our data support a model where BRX and PAX are elements of a molecular rheostat that modulates auxin flux through developing PPSEs, thereby timing their differentiation.

O-32

An Auxentric view of gene expression during plant development

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In animals, many hormones control development and metabolism by binding directly to transcription factors, thereby changing their activity as regulators of gene expression. In contrast, the signalling mechanisms for many plant hormones have at their core a process by which repressors of gene expression are degraded. One such example is auxin, which induces degradation of transcriptional repressors thereby relieving repression of auxin responsive genes. We have recently described an alternative auxin-signalling pathway mediated by the auxin response factor, ETTIN (ETT), which plays a particularly important role during the establishment of polarity in organ development. This novel auxin-signalling mechanism is fundamentally different from canonical auxin signalling as it involves a direct effect of the hormone molecule on ETT-transcription factor (TF) complexes without the involvement of protein degradation.
During plant development, ETT associates with a range of TFs in a process-specific manner to regulate gene expression. These ETT-containing complexes are sensitive to auxin and recent data from our lab has led us to hypothesise that they recruit components that control chromatin modulation to either repress or activate transcription in an auxin-dependent manner. Our preliminary data suggest that changes in chromatin state at target gene loci occur in a mechanism with reminiscence to thyroid hormone signalling and Wnt signalling in animals.

O-33

HD-ZIPII proteins coordinate a biradial-to-radial symmetry transition of auxin signalling responce during gynoecium development

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Growth and development of multicellular organisms require the establishment of symmetries during organogenesis, where cells actively divide, tissues are specified and organs reach their final shapes. The Arabidopsis gynoecium, that develops into a fruit after pollination is a complex organ, which undergoes a change in symmetry required for efficient reproduction. The gynoecium develops initially as a bilaterally symmetric structure due to its origin as two fused leaves, but immediately prior to formation of the apical style, the distal end undergoes a symmetry transition, from bilateral to radial. This bilateral-to-radial symmetry transition requires

a transitory biradial (bisymmetric) auxin distribution stage, which is mediated by two bHLH transcription factors, INDEHISCENT (IND) and SPATULA (SPT). However, the molecular mechanism that underpins these consecutive symmetry transitions remains poorly understood. Here we show that SPT genetically interacts with members of the Homeodomain-Leucin zipper (HD-ZIP) II family, such as HOMEOBOX ARABIDOPSIS THALIANA 3 (HAT3) and ARABIDOPSIS THALIANA HOMEOBOX 4 (ATHB4) to promote a biradial-to-radial transition of auxin accumulation thereby facilitating radialization of the style. Our data show that SPT together with the bHLH proteins, HECATE1 (HEC1), HEC2, and HEC3, synergistically control the expression *HAT3* and *ATHB4* to sustain the formation of a coherent radially symmetric ring of auxin-responsive cells, while restricting sensitivity to cytokinin. This mechanism allows the formation of the radial style, which is pivotal for efficient fertilization of the ovules, thus contributing to the evolutionary success of the Angiosperm phylum as well as robust production of seeds.

O-34

Cytokinins beyond plants: Understanding the evolution of the inter-kingdom signalling molecules through *Dictyostelium discoideum*

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The cytokinins (CKs) encompass a group of phytohormones that are implicated in all aspects of plant growth and development. CKs were once thought to be unique to plant taxa; however, increasing evidence suggests that these hormones are pervasive in organisms beyond the plant kingdom. CKs are ancient molecules that predate plants and until recently, research taking a concerted look at the interplay of these molecules among and between kingdoms has been limited. It is known that CKs are synthesized in a wide variety of organisms: bacteria, algae, fungi, insects, fish, and mammals, among others. This research seeks to further unveil the evolutionary significance of these ancient signalling molecules through the model organism, *Dictyostelium discoideum*. *Dictyostelium* is a cellular slime mold that diverged between the plant and animal kingdoms, retaining traits from both systems. To date, the only CKs identified in *Dictyostelium* are discadenine (DA) and isopentenyl adenine (iP), and conclusions regarding the role of CKs in this system are limited. A novel method of CK quantification in *Dictyostelium* was developed using the high-resolution, accurate mass Q-Exactive Orbitrap mass spectrometer. This method allows for greater precision and detection of trace concentrations of CKs to be compared among the developmental stages in *Dictyostelium.* This study reveals the presence of cZ, iP, iPR, iPNT, and DA. iP-type CKs were the only CKs to be found in the growth stage, with iPNT appearing in the highest levels in the supernatant (17 pmol/mL). iP and DA appeared at low levels in the mound supernatant (~0.5-1 pmol/10^6 cells) and at the highest levels in the spore matrix (iP: 27 pmol/10^6 cells; DA: 31

pmol/10^6 cells) and spore pellet (iP: 1.76 pmol/10^6 cells; DA: 6.70 pmol/10^6 cells). Furthermore, targeted metabolomic data reveals DA as one of the most dysregulated features between the developmental stages of the life cycle – being the highest in the fruiting body stage.

O-35

Longitudinal zonation and symmetries in proliferation activity of the Arabidopsis thaliana root meristem in cytokinin deficient and auxin overproducing mutants

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To date CYCB1;1 marker and cortex cell lengths have been conventionally used to determine the proliferation activity of the Arabidopsis root meristem. By creating a 3D map of mitosis distribution we showed that these markers overlooked that stele and endodermis save their potency to divide longer than the cortex and epidermis [Lavrekha et al., Plant Journal, 2017]. Cessation of cell divisions is not a random process, so that mitotic activity within the endodermis and stele shows a diarch pattern. Mitotic activity of all root tissues peaked at the same distance from the quiescent center (QC); however, different tissues stopped dividing at different distances, with cells of the protophloem exiting the cell cycle first and the procambial cells being the last. The robust profile of mitotic activity in the root tip defines the longitudinal zonation in the meristem with the proliferation domain, where all cells are able to divide; and the transition domain, where the cell files cease to divide.
We performed 3D analysis of mitosis distribution in cytokinin deficient ipt3ipt5ipt7 and auxin overproducing yuc1D to understand how changes in endogenous cytokinin and auxin levels affect the proliferation activity pattern in the root meristem. We observed decrease in the number of mitosis in yuc1D mutants and mitosis accumulation in ipt3ipt5ipt7 overall root meristem, that suggests inhibitory role of enhanced levels of both hormones on cell division. However, mitosis distribution through meristem layers indicates specific roles of the hormones in control of proliferation for different cell lineages. E.g. our data suggest much stronger inhibitory effect of cytokinin on anticlinal cell divisions in the stele, than in the outer layers.
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O-36

The role of the L-AFL transcription factors in organ patterning

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It is becoming increasingly evident that a proper auxin distribution is crucial for pattern formation during organogenesis. In the gynoecium, the female reproductive organ in flowering plants, auxin has a tightly controlled dynamism, in time and space, shaping the complex morphology of the organ. We hypothesized a role for the L-AFL network of transcription factors in the regulation of auxin distribution in gynoecium. A *lec1* T-DNA knockout allele (*lec1-2*) shows a pleiotropic effect on gynoecium morphology. *lec1-2* gynoecia are defective in style development and show a medial and/or lateral split-type phenotype. Interestingly, the *lec1-2* split phenotype seems to be regulated by light. Indeed, when plants are grown under high R/FR ratio light conditions, *lec1-2* gynoecial split phenotype is significantly enhanced. Furthermore, the *lec1-2* split phenotype is enhanced by *abi3-6* and partially rescued by *fus3-3* mutations. However, the *fus3* and *abi3* single mutants don't display any altered gynoecium phenotypes. In addition to the gynoecium phenotype, *lec1-2* also shows a decreased number of seeds per silique and an altered number of floral organs, suggesting that the *lec1-2* mutation affects organogenesis as early as in the floral meristem. To assess the involvement of auxin in the *lec1-2* gynoecium patterning defects, *DR5* promoter activity was studied. Interestingly, different *DR5* expression patterns are observed in *lec1-2* when compared to WT, suggesting that LEC1 is needed for proper auxin distribution during gynoecium development. Using yeast one-hybrid, LEC1, LEC2 and ABI3 were shown to directly bind to some auxin biosynthesis genes. However, further analyses are in progress to determine whether LEC1 is regulating auxin distribution through auxin transport, signaling or biosynthesis.
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O-37

To grow or not to grow - differential growth control

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Roots are the hidden half of plants, which certainly delayed the integration of root architectural traits into crop breeding programs. The radial expansion of the root system depends on the directional growth of secondary roots. While the main root typically grows towards the gravity, lateral root organs deviate from this and establish a distinct gravitropic set point angle (GSA) (Digby and Firn, 1995). Accordingly, the partial suppression of a full gravitropic response in lateral roots is central for the radial exploration of the soil (Rosquete et al., 2013; Roychoudhry et al., 2013). Despite the apparent importance of the dimension of the root system for plant performance, this suppressive mechanism, guiding the directional growth of lateral root, is molecularly unexplored. My lab quantitatively determined GSA of emerged lateral roots in naturally occurring Arabidopsis accession lines and have used a genome-wide association study (GWAS) to reveal novel molecular components involved in radial root expansion. Our genetic screen identified the first known anti-gravitropic component of LRs, allowing for lateral root expansion. Our biochemical analysis reveals that an identified SNP directly affects cytokinin metabolism, which is causal for the reduced bending of lateral roots to gravity. We show that asymmetric CK signaling does counterbalances auxin-dependent gravitropic growth specifically in lateral roots. Computational modeling suggests that this mechanism recapitulates radial exploration of the root system.

O-38

The tomato *BLADE ON PETIOLE* gene family and *TERMINATING FLOWER* regulate leaf axil patterning along the proximal-distal Axes

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Plants can actively shed organs in a genetically programed process termed abscission, which takes place in designated cell layers termed abscission zones (AZs). Leaf AZ initiation is part of leaf axil patterning occurs concomitant with leaf development and takes place at the boundary zone which demarcates the initiating leaf primordia from the shoot apical meristem. Subsequent growth and differentiation result in establishment of the axillary meristem (AM) and AZ along the proximal-distal axis of the leaf axil, yet the molecular mechanisms that regulate these events are poorly understood. We studied the role of the tomato *BLADE ON PETIOLE* (*SlBOP*) gene family in leaf axil patterning and leaf AZ formation using *BOP*-silenced transgenic tomato plants (artificial microRNA) as well as *BOP*-mutated lines. The tomato *SlBOP* gene family function to pattern the leaf axil along the proximal-distal axis by determining the AM position at the stem-petiole junction and regulating the development of a functional adjacent leaf AZ. Dissection of the role of the three tomato *SlBOP*s demonstrates that *SlBOP2* is the dominant gene of the three family members in regulating correct AM

positioning and AZ development in the leaf axil, but suggests that all three genes play a role in leaf axil patterning. Analysis of *terminating flower* (*tmf*) mutant plants suggest that *SlBOP*s may regulate proximal-distal pattern formation and abscission zone development in concert with TMF, a transcription factor which was previously shown to interact with *SlBOP*s.

O-39

Developmental patterning of Asteraceae flower heads

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The Asteraceae family is characterized by showy flower-like inflorescence, the flower head that mimics a single solitary flower but is composed of multiple flowers. On a head, individual flowers are organized in left and right winding spirals (contact parastichies), the number of which always follows the two consecutive numbers in a mathematical Fibonacci series (1, 1, 2, 3, 5, 8, 13 ...). Intriguingly, while *Arabidopsis* apex shows spiral numbers of 3:5 in each direction, the Asteraceae heads, such as in sunflower or gerbera, show much higher Fibonacci numbers (e.g. 34:55 or 55:89). The regularity of this phyllotactic pattern has puzzled both biologists and mathematicians for centuries. Taking advantage of micro-CT imaging, we have resolved the growth dynamics during early ontogeny of flower heads in sunflower and gerbera, two model species in Asteraceae with distinct plant architectures. We show that patterning of the flower head is initiated in the shoot apex during vegetative development, and is further propagated after floral induction, however, differently in these two species. Increasing spiral numbers are associated with expansion of the inflorescence meristem. We have produced transgenic gerbera lines with DR5 reporter indicating a major role for auxin in defining the position of emerging flower primordia. Inhibition of auxin transport by NPA treatment abolished flower primordia initiation. However, after recovery from NPA treatment, new spirals of flowers initiated, but not in Fibonacci numbers. In addition to auxin, we also found important roles for cytokinin and gibberellic acid affecting phyllotactic pattern. Our aim is to integrate the experimental data into a computational model to demonstrate how phyllotactic patterning is established in Asteraceae flower heads.

O-40

Leaf morphogenesis: from cells to shape through patterning

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How a shape arises from the coordinated behavior of cells is one of the most fascinating questions in developmental biology. Here we used the early stages of development of serrated leaves in *Arabidopsis thaliana* as a model to study the tight relation between cellular behaviour and morphogenesis. During *Arabidopsis thaliana* leaf development the fine control of cell proliferation and cell expansion sustains differential growth at the margin required for the formation of leaf outgrowth named teeth. In this model, differential growth is the result of an interplay between auxin signaling and CUC transcription factors that are involved in the maintenance of boundary domain identity. To clarify the interconnected relations between patterns of CUC TFs and auxin responses as well as the cellular events behind serrations we used time-lapse experiments on vegetative primordia of lines expressing developmental and/or auxin response reporters. Our results revealed a tight and dynamic control of differential growth at the leaf margin and the critical involvement of one of the CUC genes in the local repression of cell growth in combination with low auxin responses.

O-41

Cytokinin-dependent control of growth and development through action of the type-B response regulators

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Cytokinin affects a diverse array of growth and development processes and responses to the environment. How a signaling molecule mediates such a diverse array of outputs and how these response pathways are integrated with other inputs remains a fundamental question in plant biology. To this end, we have been characterizing the transcriptional network initiated by the type-B response regulators (RRs) that mediate the cytokinin primary response, doing so in Arabidopsis and rice. In Arabidopsis, cytokinin dependent targets were identified by a combination of chromatin immunoprecipitation-sequencing (ChIP-Seq), protein-binding microarrays, and transcriptomic approaches. Results revealed a cytokinin-dependent mechanism for type-B RR activation and binding to targets, as well as a basis by which cytokinin regulates diverse aspects of growth and development. The homeotic transcription factor *WUSCHEL* was identified as a type-B RR target, and manipulation of type-B RR levels regulated *WUSCHEL* expression as well as shooting in tissue culture. In rice, to clarify the role of cytokinin action in inflorescence development, we used the NanoString nCounter system to analyze gene expression in the early stages of rice panicle development, focusing on genes

involved in cytokinin biosynthesis, degradation, and signaling. Results point toward the involvement of specific members of these gene families in panicle development, their dynamic patterns of gene expression suggesting that subnetworks mediate cytokinin action during different stages of panicle development. Genetic analysis of rice type-B RR mutants demonstrates their key role in regulating inflorescence development, with mutant phenotypes revealing defects in panicle architecture, flower development, and grain yield.

O-42

Mechanical feedback acting on auxin distribution machinery mediates in differential cell elongation

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In dicots, immediately after seed germination apical hook forms by bending of the hypocotyl. Apical hook formation requires differential rate of cell elongation on two sides of the hypocotyl. Plant hormones such as indole-acetic acid (IAA/auxin) plays a central role in apical hook development and differential accumulation of auxin on two sides of the hypocotyl mediated by polar auxin transport is essential for apical hook development. We report on the hitherto unknown role of xyloglucans that are important constituents of primary cell wall in apical hook development. Xyloglucan mutants are defective in apical hook development. Importantly, xyloglucan mutants such as xxt1/xxt2 fail to properly establish asymmetric auxin distribution that is essential for apical hook development. The failure to establish auxin asymmetry in xyloglucan mutants is due to attenuation of auxin transporters at the plasma membrane. Although xyloglucans are present in cell walls of the all the cell layers of the hypocotyl, we show that the requirement of xyloglucans in apical hook development is confined to the epidermal cell layer. Although the role of auxin in controlling cell walls is well known, our results indicate a potential feedback from the cell wall that could act on auxin transport machinery to mediate in control of differential cell elongation.

O-43

Cytokinin regulation of cambium activity and wood formation in hybrid aspen

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Radial expansion of the stems and roots in plants derives from the activity of the vascular cambium - a meristematic tissue that contains the vascular stem cells and generates xylem (wood) on the inside and phloem on the outside. Proliferation and differentiation of the vascular stem cells in the cambium is tightly regulated to achieve an organized vascular development. A recent study from our group displayed that cambial cell division rate and biomass production can be stimulated dramatically in hybrid aspen trees through overexpression of the cytokinin biosynthesis gene, *ISOPENTENYLTRANSFERASE 7 (IPT7)*. To understand how cytokinin orchestrates the cambium activity and wood formation, we collected genome-wide profiling data from the wood-forming regions of wild-type (WT) and mutant trees with enhanced cytokinin production, and from the stem of WT trees treated with cytokinin. As a result, several new regulators of cambium development in hybrid aspen was identified. Currently we are studying the functions of these candidate genes in trees through transgenics approach. Moreover, via in silico transcript profiling we identified a *CLAVATA3/ESR-RELATED (CLE)* gene that is specifically expressed in the cambium of trees. RNAi-mediated down-regulation of this peptide hormone in hybrid aspen affected a diversity of characteristics such as lateral expansion of stems, apical growth and leaf size, suggesting it is an important regulator of tree growth and development.

O-44
The role of cytokinin signalling in rice root vascular patterning

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Cytokinin signalling proceeds through a phosphorelay in which a phosphate group is transferred from receptors to histidine phosphotransfer proteins (HPTs), and then to response regulators (RRs) which regulate expression of cytokinin responsive genes. Phosphorelay may be inhibited by the pseudo-histidine phosphotransfer proteins (PHPs), which share sequence similarity with the HPTs but have a substitution at the phosphoaccepting histidine. In *Arabidopsis* AHP6 functions in auxin-cytokinin crosstalk in many developmental contexts such as vascular patterning, lateral root development and organ initiation at the SAM. While AHP6 is the only PHP present in *Arabidopsis*, the rice genome encodes three PHPs whose functions remain unknown. In order to elucidate the roles of these OsPHPs, they were characterised primarily using root vascular patterning as a model developmental system.
While the phosphoaccepting histidine has been substituted to an asparagine in AHP6, phylogenetic analyses revealed that in rice (and all monocots) the PHPs have a substitution to a

glutamine. To determine whether this could lead to differences in inhibition of phosphorelay, in-vitro phosphotransfer assays were conducted. To understand the role of cytokinin signalling in rice root vascular patterning, the OsPHPs along with type-A OsRRs (inhibitors of cytokinin signalling) were chosen for detailed study. qRT-PCR revealed transcription of the OsPHPs (unlike AHP6) is not auxin inducible in the root tip, whereas a few of the type-A OsRRs are. Complementation assays revealed that some type-A OsRRs partially rescue *Arabidopsis ahp6* mutant phenotypes, indicating they may play a role in vascular patterning in rice. Experiments are being undertaken to fully characterise these genes, including determining expression patterns, and creating loss of function mutants through CRISPR. Together, these results suggest that some type-A OsRRs may have been recruited to fulfil the function of AHP6 in *Arabidopsis*.

O-45

IAA oxidation via DAO2 is important for floral development in *Arabidopsis*

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Auxin homeostasis is maintained by coordination of auxin biosynthesis, transport and catabolism. Recently, auxin (indole-3-actic acid, IAA) inactivation via oxidation by DEOXYGENASE OF AUXIN OXIDATION (DAO) pointed to its role in plant growth and development. However, the results have been difficult to interpret because a gene duplication resulted in two copies of *DAO*, *DAO1* and *DAO2*, in *Arabidopsis thaliana*. Functional redundancy of *DAO* has been suggested since *DAO2* appears to be expressed at very low levels and *dao1* loss-of-function line show 95% reduction in 2-oxindole-3-acetic acid (oxIAA) levels. The outstanding question is if the mild phenotypes in *DAO1* loss-of-function alleles are due to redundancy in the auxin catabolic pathway or *DAO* gene duplication. Since *DAO1* and *DAO2* are in tandem, a CRIPSR-Cas9 approach was taken to construct several independent null *dao2* and *dao1 dao2* mutants. Here we report that *DAO2* function is independent from *DAO1*. *DAO2* plays a role in regulating floral organ number, and auxin inactivation via oxidation is required for normal floral development and auxin homeostasis.

O-46

Genome-wide transcript profiling reveals an auxin-responsive transcription factor promoting adventitious root formation in rice

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Plant hormones like auxin and cytokinin play an important role during rice adventitious/crown root development but the genetic regulators working down-stream of auxin and cytokinin are not yet identified. We have identified genes regulated by auxin and cytokinin in crown tissues. Our transcript profiling studies have revealed a significantly large numbers of transcription factors regulated by auxin and cytokinin. The de-regulated expression of some genes was validated by qRT-PCR and our spatial expression pattern analysis by RNA-RNA *in situ* hybridization has identified few transcription factors specifically expressing in crown root primordia. Next, we have studied function of an auxin-regulated transcription factor from AP2 gene family during crown root formation. Its ectopic over-expression in rice causes auxin over-production/hypersentivity phenotypes, such as stem internode elongation and also induces adventitious root formation at aerial nodes, suggesting that it is sufficient to initiate root-specific developmental program at aerial nodes. Moreover, expression analysis of few known genes in the over-expression lines showed that it activates expression of auxin biosynthesis as well as auxin responsive genes and also triggers *ERF3-WOX11-RR2* pathway in a dose-dependent manner. Overall, our analysis revealed the genes downstream of auxin and cytokinin signaling pathways and identified a novel transcription factor promoting adventitious root development, possibly by regulating *ERF3-WOX11-RR2* mediated genetic network.

O-47
Cytoplasmic HSP90 proteins regulate auxin transport

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The FKBP42, TWISTED DWARF1 (TWD1), mediates through interaction with ABCB-type ABC transporters their subcellular trafficking fate and thus regulates the polar distribution of auxin. Despite previous suggestions a role of the HSP90 machinery in polar auxin transport has thus far not been addressed.
Here we identify cytoplasmic HSP90 isoforms as interacting partners of TWD1 in a proteomic co-IP approach and report co-operative TWD1 and HSP90 holdase chaperone activity *in vitro.* Interestingly, we found that treatment with the HSP90 ATPase inhibitor, geldanamycin (GA), specifically interferes with the correct subcellular localization of the same subset of ABC transporters, which are known to rely on TWD1 for targeting. Several auxin-related physiological and developmental processes were affected in non-allelic *hsp90* loss-of-function

mutants as well as in pharmacological HSP90 inhibition experiments. However, *ABCB* and *TWD1* mutation drastically increased the plant sensitivity towards HSP90 inhibitors suggesting that HSP90s act as a buffer of ABCB-mediated transport by stabilizing plasma membrane presence of redundant ABCB isoforms.
Overall we present evidence that cytoplasmic HSP90 proteins and FKBP42/TWD1 may act in concert to regulate auxin transport and thus plant development. Our findings open the possibility that plant – unlike mammalian - ABCBs qualify as a novel class of HSP90 clients in plants.

O-48

Cell intrinsic (re)establishment of PIN2 polarity in *Arabidopsis* root epidermis

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Many auxin-dependent developmental processes are driven by local auxin gradients which in turn rely on the asymmetric subcellular distribution of PIN auxin efflux carriers. What molecular mechanisms are responsible for the establishment of cell polarity in general and PIN polar localization in particular is thus a key question in auxin biology that we still know surprisingly little about. In particular, how is PIN polarity (re)established after cell division remains largely obscure.
We study the establishment of polarity in the *Arabidopsis* root meristem on the model of PIN2, a canonical marker of the apical domain of epidermal cells. Using conditionally active promoters and photoconvertible fluorescent proteins, we have generated a set of marker lines that allow us to observe the establishment of PIN2 polarity in real time. We show that PIN2 polarity establishment directly depends on endocytosis and the activity of the AGCVIII kinases PID, WAG1 and WAG2, but surprisingly does not require functional cytoskeleton. Moreover, our data suggests that in the primary root meristem, the definition of apical-basal polarity does not depend on auxin distribution or other cell-to-cell communication pathways. Since cells in the meristem undergo several rounds of cell division, this implies the existence of a cell-intrinsic mechanism to re-establish apical-basal polarity after each cytokinetic event.

O-49

TRANSPORTER OF IBA1 links cytokinin and auxin to regulate lateral root formation

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Root system architecture and consequently lateral root formation and are critical for soil exploration by plant roots, allowing for uptake of water and nutrients. Conversion of the auxin

precursor indole-3-butyric acid (IBA) to active auxin (indole-3-acetic acid; IAA) modulates lateral root formation. However, mechanisms governing IBA-to-IAA conversion have yet to be elucidated. We identified TRANSPORTER OF IBA1 (TOB1) as a vacuolar IBA transporter that limits lateral root formation, likely by sequestering IBA in the vacuole to prevent its contribution to the active auxin pool that drives lateral root formation. Moreover, *TOB1* transcripts and protein accumulate in response to the phytohormone cytokinin, which inhibits lateral root formation. The increased production of lateral roots in *tob1*mutants, TOB1 transport of IBA into the vacuole, and cytokinin-regulated *TOB1* expression suggest a mechanism linking cytokinin signaling and IBA contributions to the auxin pool to ultimately modulate root system architecture.

O-50

cis-Cinnamic acid is a novel, natural auxin efflux inhibitor that promotes lateral root formation

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Cinnamic acid (CA) is a non-toxic, naturally occurring compound which is found *in planta* both as a *trans*-(*t*)- and *cis*-(*c*)-isomer. *t*-CA is synthesized through the deamination of phenylalanine by PHENYLALANINE AMMONIA-LYASE (PAL), after which it is hydroxylated to *p*-coumaric acid by CINNAMIC ACID-4-HYDROXYLASE (C4H). These are the first steps of the core phenylpropanoid pathway that leads towards a plethora of secondary metabolites, such as flavonoids, stilbenes, tannins and monolignols. *c*-CA is the photo-isomerization product of *t*-CA.

Cinnamic acid has since the 1930s been historically defined as a bioactive molecule with auxin-like effects. However, it remained unclear whether the *cis*- or *trans*-isomer showed bioactivity. Recently we demonstrated that *t*-CA is inactive, consistent with its primary role as an intermediate in the general phenylpropanoid pathway. In contrast, its *cis*-isomer is biologically active and acts as a natural inhibitor of cellular auxin efflux, promoting lateral root formation. When grown on *c*-CA-containing medium, an evolutionary diverse set of plant species were shown to exhibit phenotypes characteristic for high auxin levels, including inhibition of primary root growth, induction of root hairs, and promotion of adventitious and lateral rooting. Our work revealed a novel mechanism on how plants may regulate auxin levels and adds a novel, naturally occurring molecule to the chemical toolbox for the studies of auxin homeostasis.

O-51

ABCB transporters: why is boring non-polar plasma membrane exclusion necessary for long distance polar auxin transport?

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Polarized auxin streams that function in embryogenesis, organogenesis, and other aspects of plant development are fully described by nonpolar lipophilic and anionic uptake coupled to polarized cellular efflux mediated by PINFORMED carrier proteins. These processes are motivated by plasma membrane chemiosmotic potentials. The presence of the fundamental cellular components that mediate these polar auxin transport streams are sufficient to assure basic development. All other transport processes described to date elaborate these fundamental mechanisms. However, long distance polar auxin transport streams depend on the presence of ATP Binding Cassette Subclass B (ABCB) active transporters generally associated with multisubstrate specificity and exclusion of hydrophobic anions from plasma membrane leaflets. Simple models of transport developed in Arabidopsis seedlings implicate ABCB exclusion mechanisms in limiting polar auxin streams to the central vascular cylinder of hypocotyls and roots. Such a function would require limited substrate specificity for efficacy. Evidence of the importance of these transporters is more evident in mature plants, where vasculature is more diverse and a boundary function is not supported by transcript and protein localization. Instead, the primary function of ABCB transporters in mature tissues appears to be prevention of auxin reuptake in apical and dividing cells adjacent to sites of loading into transport streams. The specific ABCB transporters contributing each of these functions have been isolated and identified in Arabidopsis. An analysis of their distribution and activity suggests that membrane exclusion is a generalized function and that gene duplication allowed for greater auxin substrate specificity in a limited subset of the subfamily. This new functionalization resulted in their emergence as primary factors in maintenance of long distance polar auxin streams, while other isoforms may exhibit more diverse function.

O-52

Phosphorylation control of PIN auxin efflux carriers by D6 PROTEIN KINASES and associated proteins

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Auxin transport from cell to cell and within a plant had for a long time been inferred solely based on the polar distribution of auxin transporters such as the PIN-FORMED auxin exporters. We have recently shown that PINs require activation by protein kinases, such as the D6 PROTEIN KINASE (D6PK). I will describe the biochemistry of D6PK and novel mechanisms that are required for targeting functional D6PK to the plasma membrane where D6PK phosphorylates and activates PINs. Using phosphosite-specific antibodies, we have obtained evidence that PIN phosphorylation coincides with the presence of proteins sensitive to the trafficking inhibitor Brefeldin A, a feature of D6PK but not other protein kinases previously suggested to regulate PINs. At the same time, these findings challenge mechanisms explaining PIN polarity control by such phosphorylation events. Finally, our recent studies have allowed identifying other proteins of previously unknown biochemical function in D6PK-regulated and, at least in part, PIN-dependent auxin transport regulation. The most recent and relevant progress will be presented.

O-53

Alternative splicing of PIN auxin efflux carriers

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Alternative splicing is a posttranscriptional modification, which can significantly enhance a gene coding potential. According to transcriptome sequencing, more than half of Arabidopsis genes undergo this process. Alternative splicing has been described in several proteins involved in auxin dependent processes and also in some auxin carriers of the PIN-like family (PINs). PINs are polarly localized on the plasma membrane and drive crucial developmental decisions by distribution of auxin maxima throughout the plant.

We have selected splicing of PIN4 and PIN7 as a model for deciphering importance of alternative splicing in hormonal pathways. Both genes are identically processed into two relatively conserved transcripts (termed a and b). We designed PIN7a/b splicing fluorescent protein-based sensors which revealed that both isoforms are expressed with similar quantities in similar tissues. Whereas PIN7a and b transport auxin with similar capacity in the tobacco cell lines, their functional roles seem to be distinct. We observed a significant difference in intracellular trafficking properties where PIN7a showed more rapid dynamics inside cell than PIN7b. PIN7a is able to almost fully complement various mutant phenotypes tested, while PIN7b showed only partial phenotype rescue in this respect. The difference between isoforms complementation was especially notable in quick-response tests (such as phototropic or gravitropic bending). We therefore conclude that there are probably two populations of PIN7 protein. PIN7a, which is dynamic and dedicated to quick response to exogenous stimuli, and PIN7b, which represents more static route, likely purposed for maintaining stable auxin flow.

O-54

Hormonal control of shoot branching

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The degree of shoot branching is an excellent example of plant developmental plasticity. Branches develop from axillary buds established in the axil of each leaf. The buds can remain dormant, or activate to produce a branch, and this switch is regulated by diverse endogenous and exogenous factors, allowing plants to tune their development according to the prevailing environmental conditions. A growing body of evidence suggests that a central regulator of this process is the shoot auxin transport network. The self-organising properties of this network mediate competition between buds, with reinforcement, balancing growth across the shoot system. In addition, local expression of the BRC1 gene in the bud also affects its ability to activate. This dual-mode system is likely to provide robustness to the bud activation switch. Interestingly, in Arabidopsis, the branch regulating hormones, strigolactone and cytokinin, each appear to regulate both the auxin transport network and BRC1 expression.

O-55

Digging for novel regulators of rooting at the crossroad of Auxin and Jasmonate crosstalk

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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. In order to identify genes involved in the control of AR formation we used Arabidopsis as a model. We have previously shown that AR formation is controlled by a complex crosstalk between auxin (IAA) and Jasmonate (JA). In this study, we will describe our strategy to identify downstream targets of JA signaling. We performed RNA-seq using a JA signaling mutant, which is affected in AR but not LR development. The data analysis highlighted several potential downstream regulatory genes. we also identified a potential feedback loop between JA signaling and IAA homeostasis. In addition, several cytokinin (CK) perception and signaling genes were differentially expressed downstream of JA signaling, highlighting a possible JA-mediated IAA-CK crosstalk during AR formation.

O-56

Interaction of auxin and cytokinin in the specification of vascular pattern in diverse species.

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Pattern formation is typically controlled through the interaction between molecular signals within a given tissue. During early embryonic development, roots of the model plant, Arabidopsis thaliana, have a radially symmetric pattern, but an asymmetric input of the hormone auxin from the two cotyledons forces the vascular cylinder to develop a diarch pattern with two xylem poles. Molecular analyses and mathematical approaches have uncovered a regulatory circuit centring around auxin-cytokinin crosstalk that propagates this initial auxin asymmetry into a stable cellular pattern. The diarch pattern seen in Arabidopsis, is relatively uncommon amongst flowering plants, with most species having between three and eight xylem poles. In this talk, I will present a multiscale mathematical modeling approach to demonstrate that this regulatory module does not rely on an asymmetric auxin input to specifying vascular pattern. Instead pattern can organize dynamically, with final pattern being dependent upon spatial constraints and growth. The predictions of our simulations compare with experimental observations of xylem pole number across a range of species and in transgenic systems in Arabidopsis where we manipulate the size of the vascular cylinder. Through considering the spatial constraints, our model is able to explain the diversity seen across flowering plants.

O-57

Hormones interaction during flower and fruit development in tomato

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Hormones play a pivotal role in most physiological processes in plants. The aim of this research was to elucidate the role of plant hormone from different chemical families and interaction between hormones during flower and fruit development. We used transcriptome analysis (RNASeq) to identify hormone-related genes using Illumina digital gene expression technology and by comparing the hormone metabolome using UPLC-ESI-MS/MS. The gene expression and metabolome were determined in different flower and fruit organs during different developmental stages. In order to better understanding the interaction between different hormones we used two methodologies: we follow cytokinin response factor (TCS::VENUS) and auxin response factor (pIAA::mRFP) expressed in the same plants during flower and fruit develpment. We documented the expression of the two and co-expression in shoot apical meristem and during flower organ and fruit development. We also determine the hormone related gene transcriptome and hormonal metabolome in hormone biosynthesis mutants (*GA20ox*, *CKX3* and *flacca*). The hormonal related gene expression and metabolome data shows differential hormone expression and co-expression between flower organ, fruit organs, tomato mutants during tomato flower and fruit development. The RNASeq data and hormone content analysis shows a complicated spatial and temporal expression pattern of the hormone-related gene expression and metabolome during flower organ and fruit development. Furthermore, the TCS::VENUS and pIAA::mRFP spatial and temporal expression patterns that different flower developmental process are under a complex control of both hormones. The mutant transcriptome and metabolome reveal tight negative and positive interactions between the different hormones chemical groups. These dynamic expression patterns might point on the important role of hormone homeostasis and interaction during flower and fruit developmental events.

O-58

The involvement of endogenous plant hormones in the regulatory network of fatty acid biosynthesis in soybean seed

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The activities of phytohormones during the reproductive phase have been partially clarified in seed physiology while the biological role of plant hormones in oil accumulation during seed development has been investigated in part only. In this research, fatty acid (FA) contents and hormone profiles, including abscisic acid (ABA) and cytokinins (CKs) of seed samples in four

different stages and comparing six soybean varieties have been investigated in order to examine the hypothesis that the endogenous plant hormones play important roles in FA production in soybean seeds. The FA contents increased significantly during this period while the hormone concentrations gradually declined towards the seed physical maturation. However, the interactions between FA contents and hormone profiles were complex and went beyond linear correlations. Hormone metabolism in the earlier stages of seed maturation period demonstrated numerous robust relationships with FA accumulations, as derived from several simple and multiple regression models in the determination of different FA contents. Evaluation of the effects of exogenous ABA and *trans*-Zeatin (*tZ*) on FA biosynthesis has revealed that ABA appears to be involved in the accumulations of unsaturated FAs while *tZ* participated in the synthesis of saturated and unsaturated FAs. Notably, the alterations of FA synthesis differ according to what exogenous hormone concentrations could be used. Moreover, metabolomics analysis of seeds derived from exogenous the hormone treatments was also obtained using a high resolution mass spectrometry, Thermo Q-Exactive. The metabolomics profile has help to shed more light on the effect of hormones on fatty acid production and seed metabolism in general.

O-59

Auxin and cytokinin synergism regulates secretory pathway to steer elongation growth.

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Plants as non-locomotive organisms constantly integrate varying environmental signals to flexibly adapt their growth and development. Local heterogeneities in water and nutrients availability, sudden changes in temperature, light or other stresses trigger dramatic changes in the plant growth and development. Plant organs such as primary and lateral roots, hypocotyls and stems can rapidly react to the environmental perturbations by modulating their growth kinetics. Role of hormonal signaling cascades as essential endogenous translators of these exogenous signals in plant adaptive responses is well established. Using a genome wide transcriptome profiling we identified a novel component of auxin and cytokinin cross-talk, *SYNERGISTIC AUXIN CYTOKININ 1* (*SYAC1*) whose expression in roots is strictly dependent on both hormonal pathways. Detailed functional characterization reveals that SYAC1 acts as a developmentally specific regulator of the secretory pathway to control deposition of cell wall components and thereby rapidly fine tune elongation growth of plant organs.

O-60

Mutations in tetrapyrrole biosynthesis pathway uncouple nuclear *WUSCHEL* expression from *de novo* shoot development in *Arabidopsis thaliana*

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Plant *de novo* organogenesis in tissue culture systems has long been exploited to study the plasticity of pluripotency and auxin-cytokinin interaction. External application of high cytokinin-to-auxin ratio in cultured medium stimulates greening of calli and promotes nascent shoot apical meristem (SAM) formation. The stem cell niche in SAM is maintained by a negative feedback loop between CLAVATA-WUSCHEL (WUS) signaling. Cytokinin being known to induce *WUS* expression, the capacity of *de novo* shoot development is largely coupled to *WUS* activity. However, the molecular mechanism of *WUS* expression remains obscure. Here we provide a novel regulatory mechanism of *WUS* expression during *de novo* SAM formation that is affected by the altered tetrapyrrole metabolism catalyzed in the plastid. Loss-of-function mutations in *Mg-CHELATASE* subunits (*chld, chli1* and *chlh/gun5)* and its regulator (*gun4), Mg-PROTO IX MONOMETHYLESTER CYCLASE* (*crd1)*, *HEME OXYGENASE*(*hy1/gun2)* and *PHYTOCHROMOBILIN SYNTHASE* (*hy2/gun3*) result in elevated *WUS* expression but the shoot regeneration efficiency is decreased whereas loss-of-function mutation in *PROTOPORPHYRIN IX FERROCHELATASE 2* (*fc2*) exhibits compromised *WUS* expression with reduced number of shoots when mutant root explants are cultured on shoot induction medium. The fact that all loss-of-function *gun* mutant alleles defects in tetrapyrrole metabolism examined in this study as well as mutants defect in the other Mg-chelatase subunits show uncoupled *WUS* expression from shoot regeneration suggests that plastid-to-nucleus communication takes place during *de novo* organogenesis for fine-tuning a nuclear gene expression.

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O-61

Light controls cytokinin signaling via transcriptional regulation of constitutively active histidine kinase CKI1

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Crosstalk between light and cytokinin response is known for decades. However, the underlying molecular mechanism remained unclear. Via the automated microscopy-based screen we identified *long pale hypocotyl (lph)* mutant exhibiting disturbed spatiotemporal expression pattern of *CYTOKININ INDEPENDENT-1 (CKI1)*. *CKI1* encodes for constitutively active sensor histidine kinase acting via MSP and controlling female gametophyte and vascular tissue formation in Arabidopsis. We found out that *lph* is a new allele of *HEME OXYGENASE 1* (*HY1*),mediating biosynthesis of phytochromobilin, a cofactor of the photoconvertible light receptors phytochromes. Following analysis confirmed light-dependent regulation of *CKI1* and dual (both positive and negative) role of light receptor phytochromeA (phyA) in the control of spatiotemporal specificity of *CKI1* expression. The phyA-regulated transcription factors PHYTOCHROME INTERACTING FACTOR 3 (PIF3) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) were found to bind the *CKI1* promoter, thus supporting direct regulation of *CKI1* by phyA-mediated signaling. We also show that changes in the expression of *CKI1* in the light-deficient mutant *lph,* as well as impaired light signalling in loss-of-function mutants *phyA, phyB* and *phyAphyB* result in disturbed MSP activity, altered sensitivity to cytokinins and developmental aberrations that were previously shown to be associated with cytokinin and/or CKI1 action. Finally, our study revealed the role of phyA-regulated *CKI1* activity in the control of hypocotyl elongation and hook formation during skotomorphogenesis. We propose that the light-dependent regulation of *CKI1* provides a conceptually novel mechanistic link between light- and MSP-mediated regulations of plant development.
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O-62
Auxin and ABA signaling coordinate branching responses to light signals

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Shoot branching is an important plant architectural characteristic, affecting plant fitness in natural environments and productivity in agricultural crops and pastures. Intrinsic genetic programs are major determinants of branching, but it is also known that environmental signals regulate this central aspect of plant form, with light signals exerting profound effects. For instance, ongoing research has shown that the ratio of Red light to Far Red light (R:FR) which is perceived by the phytochromes, modulates branching. Reduced R:FR signals impending competition from neighboring plants and elicits the shade avoidance response, which includes decreased branching. Systemic auxin in the polar auxin transport stream is understood to play a central role in suppressing shoot branching, though its mechanism of action is incompletely understood. While low R:FR has previously been shown to rapidly increase IAA accumulation in young Arabidopsis seedlings, this effect is not apparent in stems of mature plants subjected to chronic low R:FR. Rather, repeated exposure to low R:FR elevates auxin signaling independently of IAA abundance, which results in the suppression of axillary bud growth. Auxin does not operate alone however, as the inhibition of bud development is also dependent on bud- localized ABA, among other factors. Interactions between auxin and ABA occur at different levels to coordinate branch development. Light signaling components downstream of phytochrome, including the phytochrome interacting factors PIF4 and PIF5, modulate auxin and ABA homeostasis and signaling to evoke appropriate branching responses to critical environmental information.

O-63

Organic electronic ion pumps and their plant hormone delivery repertoire

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The organic electronic ion pump (OEIP) provides flow-free and accurate delivery of small organic and inorganic compounds at high spatiotemporal resolution. We have shown previously that the OEIP is capable of delivering biologically relevant amounts of the plant hormone auxin to *Arabidopsis thaliana* roots *in vivo.*

We have now developed a second generation of OEIP with narrower glass tube and a clearly visible delivery channel, enabling for much more precise positioning of the pump to various *A. thaliana* root zones.
We demonstrate the use of OEIP for various plant related compounds and hormones delivery to buffered media or root tissues of *A. thaliana* seedlings *in vivo*. Isopentenyladenine (iP), *trans*-zeatin, 6-benzylaminopurine (BAP), abscisic acid, jasmonic acid, ACC (ethylene precursor), L-glutamine, GABA, melatonin, serotonin, brefeldin A, auxinole and endosidin 3 deliveries were confirmed by mass spectrometry measurement. Our results provide a strong starting point for an improved and highly versatile technology enabling direct, rapid and dynamic interaction of various plant hormones and other chemicals with regulation systems of plants.

O-64

Boron, auxin and cytokinin during reproductive development in maize

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Boron is an essential micronutrient required for proper plant growth. Boron crosslinks subunits of pectin, called Rhamnogalacturonan II in the cell wall, therefore giving the cell wall its stability. Roles of boron beyond the cell wall have been hypothesized, but up to now have not been shown. Plants have to take up boron from the soil either via passive uptake or through active uptake with the help of transporters.
We have identified the *tasselless1 (tls1)* gene in maize as the co-orthologue of the Arabidopsis boron importer NIP5;1. Since the *tls1* mutant cannot actively take up boron out of the soil, it is inherently boron deficient. By using this mutant, we found that one of the earliest symptoms of boron deficiency is a reduction in meristem size. Depending on the boron availability in the soil, this leads to vegetative and/or reproductive defects, which can all be rescued by boron supplementation. Under low boron conditions *tls1* dies at the seedling stage, while under higher boron conditions *tls1* either completely lacks the tassel (male inflorescence) and the ear (female inflorescence) or both structures are severely reduced. To understand what causes the reduction in meristem size in *tls1*, we are studying the involvement of boron in meristem maintenance pathways as well as in hormone pathways regulated by cytokinin and auxin. We are analyzing double mutants between *tls1* and meristem mutants and with mutants involved in auxin/cytokinin biosynthesis and signaling. These analyses are combined with confocal and fluorescence microscopy of marker genes such as *ZmWUSCHEL:RFP*. Our analyses indicate that boron levels are important for maize meristem maintenance and point to a potential interaction between boron, auxin and/or cytokinin.
Our studies will aid in understanding the role(s) of boron in plant development on a cellular level and might shed light on roles of boron beyond the cell wall.

O-65

Auxin, cytokinin, strigolactones and sugars - roles in shoot branching

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Shoot branching occurs due to the regulation of the outgrowth of axillary buds which are embryonic shoots in the axil of leaves. Long-distance signaling is central to this regulation and mainly involves strigolactones, cytokinins, auxin and sugars. The sugar role may be at least partly due to sugar signalling and to involve trehalose 6-phosphate. It also appears that the growth of axillary buds from a state of very slow growth or dormancy, to sustained growth involves a number of stages during which the emerging shoots show differential sensitivity to growth stimulus and inhibition. For example, there are substantial differences in responses to different hormones at different periods after shoot tip removal. This could be due to differences in hormone signaling and downstream responses as well as due to changes in the vasculature of the growing buds. We will present our latest unpublished findings on the interaction of signals during bud outgrowth. In addition to providing a new mechanism for how plants respond to shoot tip removal, this work is provides a better understanding of how plants achieve diverse architecture in response to the environment.

O-66

Altered day-night rhythms cause a new type of abiotic stress revealing crosstalk between cytokinin, jasmonic acid and the circadian clock

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Plants have to adapt to a regular rhythm of day and night. Recently it was found that prolongation of the light period on the expense of the dark period causes a novel type of abiotic stress, coined altered photoperiod stress (Nitschke *et al.*, Plant Cell 2016; Trends Plant Sci. 2017). Cytokinin (CK)-deficient plants show a particularly strong stress response which consists of the induction of stress and cell death marker genes, an increased jasmonic acid concentration and an oxidative burst during the night following the prolonged light period. These events are followed by a significant reduction of photosynthetic efficiency and the formation of leaf lesions during the next day.
CK-deficient plants and certain clock mutants shared a lowered expression or impaired functioning of the clock´s central oscillator genes *CCA1* and *LHY*, which indicated that a functional clock is essential to cope with an altered photoperiod. Erroneous transcriptional clock output caused enhanced expression of JA synthesis genes, leading to an increased JA content. Genetic experiments indicated that JA is causal for PCD in CK-deficient plants as introgression of the JA synthesis mutant *jar1* strongly alleviated the stress phenotype. However, recent advances revealed that not JA itself, but rather a yet unknown function of JAR1 is required to establish the stress phenotype. A possible role of reactive oxygen species (ROS) was suggested by the induction of ROS marker genes and the occurrence of an oxidative burst. Detailed analysis of the ROS scavenging system has revealed that stress-responsive genotypes have strongly altered catalase and apoplastic peroxidase activities in comparison to non-responsive plants. Additional molecular and genetic analyses of CK, JA, clock and ROS mutants and their hybrids will be discussed. Together, this work has revealed a novel CK-regulated stress response pathway and crosstalk of the CK system with the circadian clock, JA and the oxidative stress response.

O-67
Light control of leaf flattening

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Flat leaf development is a highly conserved trait in higher plants. This allows efficient light interception, while optimizing gas exchange. Given its importance for plant growth, leaf development is a highly controlled process. Auxins play an important role, starting with the establishment of the adaxial/abaxial polarity until leaf expansion, affecting cell differentiation and growth. While a flat leaf can achieve maximal light interception, curved leaves absorb light coming from different directions and are less susceptible to photoinhibition. Accordingly, leaf shape and position is controlled by light signals from the environment. The blue light receptors phototropins (PHOT) have a major role, as shown by the curved leaf phenotype in the double *phot1phot2* mutant. Also, several PHOT signaling mutants show leaf-flattening defects with a particularly striking phenotype in *pks3* null alleles. The red and far-red light receptor phyB can also control leaf development, interacting with PHOT action. The objective of this

work is to disentangle the signaling mechanism underlying PHOT control of leaf blade curvature and how these interact with auxin signaling to finely tune development in response to light conditions. To this aim, we first studied the timing of phototropin action during leaf development, finding that they have a major role during the leaf expansion phase. Second, based on the knowledge we have on PHOT control of hypocotyl bending towards unidirectional light we set out to analyze whether these mechanisms were conserved in leaf development. Similarly, perception of a light gradient throughout the leaf seems important for leaf shape determination. Finally, we studied the auxin signaling status throughout leaf development analyzing fluorescent and histochemical reporters and mutant plants phenotypes in various light conditions. Despite their minor role in hypocotyl bending, AUX/LAX transporters have an important role in PHOT-controlled leaf flattening.

O-68

Cytokinin-stress connections and the role of CRFs

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Cytokinin is a plant hormone well-known to be connected to plant growth and developmental processes as well as more recent links to abiotic stress. We have examined the connection between abiotic stress and cytokinin using parallel transcript expression and cytokinin level measurement approach in both Arabidopsis and tomato. Plants treated with oxidative or salt stress had their cytokinin levels measured by LC/MS revealing changes in different cytokinin forms (active, transported, conjugated) unique to each stress. In parallel, RNA was extracted from these plant to perform transcriptome (RNAseq) analyses and determine differentially expressed (DE) genes affected by each stress. Cytokinin Response Factors or CRFs are a group of transcription factors connected to both cytokinin and abiotic stress. We examined the role that CRFs play in these processes using CRF mutant and knockdown lines. Transcriptome and cytokinin measurements were also performed on these CRF lines, and the data compared to WT. We found general increases in several cytokinin forms under salt stress conditions that appear regulated by CRF2 (a Clade I CRF). In contrast we found under oxidative stress conditions cytokinin levels are reduced and appear regulated by CRF5 and CRF6 (Clade III CRFs). Physiological examinations of CRF lines under stress conditions confirming these results has also been performed. Furthermore, CRFs have deeply distinct evolutionary lineages

throughout Angiosperms, designated as CRF Clades I to V. A MEME-based analysis of promoter elements from each CRF Clade has revealed conserved clade specific cis-elements related to stress responses and other hormones.

O-69

Cytokinins mediate resistance and determine the bacterial biocontrol activity against hemibiotrophic bacterial pathogens

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Considering future demands in plant protection and restrictions in the use of classic pesticides, the development of alternative strategies is a major goal. Biological control of diseases by beneficial microbes offers therein a high potential for integrated plant disease management.
Cytokinins (CK) are phytohormones that are known for long time to be involved in various regulatory processes of plant physiology and development, but have only recently been shown to modulate plant immunity. Complementary experimental approaches, including autoregulated CK synthesis in response to pathogen infection, showed that CK enhance resistance against the virulent hemibiotrophic pathogen *Pseudomonas syringae* in tobacco. The CK-mediated resistance strongly correlated with an increased level of bactericidal activities and was shown to depend on the two major antimicrobial phytoalexins in tobacco, scopoletin and capsidiol. The specificity of the underlying mechanism is evident from a differential effect of the cis- and trans-isomers of zeatin. The integration into the phytohormone defense network is evident from the involvement of salicylic acid and the negative interference of abscisic acid.
The mechanism of CK-triggered immunity was also shown to be the basis for the biocontrol activities of a beneficial *Pseudomonas fluorescence* strain that had been identified based on its growth promoting activity. Complementary gain- and loss-of-function approaches with the host plant and the biocontrol strain identified the microbial CK production as a key determinant of the protection of *Arabidopsis* from the bacterial pathogen *P. syringae*. Functional cytokinin perception in combination with salicylic acid biosynthesis and signalling were essential to fully establish this biocontrol effect. The implications for the coevolution of host plants and CK-producing agonists and pathogens and the application in agriculture to enhance both pathogen resistance and abiotic cross tolerance are discussed.

O-70

Protein and Gene regulatory network involved in hormone-dependent nutrient sensing

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To optimize their growth and development plants are able to sense their environment and to modulate their development. Beside its role as nitrogen (N) source, nitrate (NO_3^-) is a signaling molecule involved in different physiological responses. The effect of nitrate on lateral root development has been described with a local and a systemic component.
We used a combination of functional screen in heterologous expression systems (Xenopus oocytes, COS cells, Yeast), transcriptomics, hormone quantification and root development analysis to identify the gene and protein regulatory networks involved in this nitrate-dependent signaling.
An important protein in the plant response to nitrate is NRT1.1/NPF6.3 a membrane protein initially characterized as a nitrate transporter. Its role in nitrate signaling is mediated by its nitrate-dependent auxin transport capacity. CIPK23, a protein kinase and CBL9, a calcium sensor, are known to be part of the NRT1.1/NPF6.3 macromolecular complex regulating both transport and sensing. Using a functional screen in xenopus oocytes, we have identified a protein phosphatase belonging to this protein regulatory network. The role of this phosphatase in the regulation of nitrate transport and sensing will be presented.
To gain insight into long-distance N-signaling we combined split-root experiments with the analysis of CK mutants, hormone profiling, gene expression analysis in roots and shoots, NO_3^- uptake assays and growth measurements, in *Arabidopsis thaliana*. The role of trans-zeatin in systemic nitrate response will be presented.
Other approaches initiated to identify other components of nitrate-hormone crosstalks will be presented.

O-71

Hormonal response of selected Brassica crops under drought stress

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Drought stress is one of the most severe abiotic stresses that may affect growth, yields, and product quality of Brassica crops, particularly in Mediterranean, semi-arid and arid

environments. Depending of their tolerance, plants have developed different mechanisms to cope with drought at morphological, physiological, cellular and molecular levels. Selected Brassica varieties: Chinese cabbage (*B. rapa* ssp. *pekinensis*), white cabbage (*B. oleracea* var. *capitata*), and kale (*B. oleracea* var. *acephala*) were evaluated to drought with particular focus to the correlations between drought tolerance and phytohormones' responses (ABA, auxin, brassinosteroids, cytokinins, jasmonates, and SA). Based on the physiological and biochemical stress markers (photosynthetic performance, proline, lipid peroxidation, protein oxidation, and antioxidant enzymes activities) drought tolerance appeared as follow: Chinese cabbage <white cabbage< kale. As expected, stress hormones (ABA, jasmonates, SA) were found to be strongly correlated with drought tolerance/sensitivity in selected Brassica crops. The most prominent increases in the level of ABA and jasmonates (JA and JA-Ile) were obtained in more sensitive varieties, while SA increased significantly only in more tolerant kale in comparison to corresponding controls. Considering brassinosteroids, a significant increase of typhasterol (TYP) was found in more tolerant kale, while castasterone (CS) and brassinolide (BL) were significantly increased in drought sensitive *B. rapa.* Increase in the level of indole-3-acetic acid (IAA) was obtained in more tolerant kale in comparison to others. The CK biosynthesis was prominently altered, including CK nucleotides, ribosides and free bases. Free bases *c*ZR and *c*Z were highly increased in kale in comparison to others. Correlations among phytohormones and biochemical stress markers were discussed based on Pearson's coefficients and principal component analysis (PCA).

O-72

Cell surface TMK mediated transcriptional auxin signaling in plants

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Auxin plays diverse and profound roles in plants, regulating essentially all aspects of plant growth and development. Yet how auxin achieves such functional diversity and specificity remains poorly understood. Auxin is perceived by TIR1/AFB receptor that regulates a nucleus-based transcriptional auxin signaling. This is to date the only known auxin signaling in regulating gene transcription. We uncovered a cell surface Transmembrane Kinase (TMK) based transcriptional auxin signaling that is required for the differential growth to form the apical hook in Arabidopsis. We found that high auxin levels stimulate the protein cleavage of TMK to negatively regulate gene expression and spatially inhibit growth. This is the first to explain the mechanism of high auxin-mediated growth inhibition and allows the differential interpretation of the distinct cellular auxin concentrations enabling the complex developmental outputs of this versatile signaling molecule.

O-73

Mathematical modeling of the effects of chilling stress on *Arabidopsis thaliana* root stem cell niche

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In Arabidopsis, chilling stress (24 h under 4°C) leads to DNA damage predominantly in root stem cells and their early descendants. However, only newly generated/differentiating columella stem cell daughters (CSCDs) preferentially die in a programmed manner. Inhibition of the DNA damage response in these CSCDs prevents their death but makes the stem cell niche more vulnerable to chilling stress. We studied the protective effect of CSCD death on the stem cell niche under chilling stress in a mathematical model.
As a readout, we analyzed the expression levels of DR5::GFP, PIN::PIN-GFP, and PIN::GUS at 4°C and 22°C. Chilling stress significantly alters expression of auxin transporters and lead to decrease in auxin response in the QC. Consistent with the experimental findings, in silico analysis showed that differential changes in the expression of PINs led to a new steady-state equilibrium of auxin distribution in chilling-stressed root, despite an overall decline in auxin levels. In this new steady state, however, the division of CSCs caused a loss of auxin maximum in the QC, which could be restored only if the death of newly generated CSCDs occurred. By contrast, CSC division at normal temperature had no effect on the maintenance of auxin maximum in the QC. We thus concluded that CSCD death was the strategy used by the root to sustain the auxin maximum.
In agreement with our model prediction that CSCD death increases the auxin concentration in the QC, roots with chilling stress-induced CSCD death displayed a higher DR5::GFP and WOX5::GFP expression in the QC than those without. Together, our findings indicate that chilling stress-induced death of CSCDs results in an increase of auxin levels in the root stem cell niche, which helps prepare the root to withstand the accompanying environmental stresses and to recover faster when returned to optimal temperatures.
The work was supported by RSF (project No 17-74-10102).

O-74

Auxin homostasis in tomato and *Arabidopsis* under heat stress

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The steady increase in temperature causes worldwide losses in agriculture, therefore heat stress is a problem in many areas in the world. Tomatoes are a major horticultural crop worldwide. Their yields are maximized in climates with moderate temperatures, whereas high temperature exposure limits tomato flower development, fruit set, and thus productivity. Experimental evidence indicates that reduced fruit set at high temperatures results from inhibited pollen development, anther release, and pollen viability reduction correlating with altered metabolism of the plant hormone auxin. We have therefore set out to analyze the auxin metabolome and transcriptome in tomato flower and fruit organs to identify candidates for alteration of auxin homeostasis. Among the strongly differentially regulated genes, four GH3 genes encoding auxin conjugate synthetases were specifically upregulated in male flower organs and gametophyte during flower development of tomato. We cloned these for heterologous expression in *Escherichia coli* to determine their *in vitro* activities. One highly regulated gene encoding an amino acid conjugate hydrolase has also been investigated in terms of activity. Since the generation of transgenic tomato plants is more time consuming than transformation of Arabidopsis, we have created transgenic lines overexpressing IAA amino acid conjugate hydrolases and GH3 genes from different sources and started to analyze these for possible heat stress tolerant phenotypes. Results from work with these transgenic plants will be presented.

O-75

Auxin drives angle-dependent gravitropic behaviour in the root.

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Gravitropism, the ability of plants to adapt their growth according to gravity, is a fundamental regulator of plant form. Historically, research on gravitropism has been dominated by two concepts. The first, known as the starchstatolith hypothesis, provides a physical basis for gravity perception: the sedimentation of dense, starch-rich plastids called statoliths within specialized cells gives information on orientation in the gravity field. The second, the Cholodny-Went hypothesis, provides the physiological basis for graviresponse: asymmetric accumulation of the hormone auxin to the lower side of the gravistimulated organ drives tropic growth. Recent work on gravity-dependent non-vertical growth in lateral roots and shoots has highlighted the importance of a third, even older concept: that the rate of gravitropic bending is dependent on stimulation angle. This phenomenon was first described by Sachs in his Sine Law of gravitropism.
To explore the mechanistic basis of angle-dependence, we developed a mathematical model of graviresponse based on high-throughput data of Arabidopsis primary roots reorientation kinetics. We show that gravitropism is a fundamentally noisy process that is best treated as a stochastic system exhibiting fast angle detection and relatively slow response, with limited to no hysteresis. Further, we demonstrate that angle-dependent gravitropic response is reflected in both PIN protein asymmetry in gravity-sensing columella cells and auxin flux to the lower half of the root, in a manner predicted by our stochastic model. Our work establishes the mechanistic interconnection of statolith-based graviperception, Cholodny-Went-based graviresponse, and gravitropic angle-dependence, providing a coherent framework for understanding the biophysical and molecular basis of gravitropism.

O-76
Cytokinin-induced priming againts biotic stress

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Priming is the indirect enhancement of the immune response of plants to pathogens. Compared to unprimed plants, the immune response from primed plants, upon pathogen attack, is much stronger. Recent research in *Arabidopsis thaliana* has shown that the plant hormone cytokinin has a priming effect against biotrophic pathogens, a phenomenon we call cytokinin-induced priming. The molecular mechanisms behind priming remains largely unknown, although recent studies have indicated that chromatin modifications, such as acetylation/de-acetylation, may

play a role. Here, we show that cytokinin perception is necessary for the action of other chemicals that have known priming activity. Further, using a gene expression meta-analysis, we identify a histone deacetylase (HDAC) whose expression is regulated by cytokinin. We show through genetic analyses that this HDAC functions as a negative regulator of cytokinin-induced priming. Chromatin mapping using Assay for Transposase-Accessible Chromatin using sequencing (ATAC-Seq) indicates that priming by cytokinin involves differential regulation of genes involved in nitrogen assimilation, metabolism, and amino acid transport which alters the susceptibility of the plant to pathogens. We propose a model in which cytokinin-induced chromatin regulation of overall nitrogen status in plants functions as a new and general mechanisms of defense priming against biotic stress

O-77

Cytokinin in thermomorphogenesis

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Temperature is a key environmental factor regulating plant growth, development and seasonal behaviour. While fundamental mechanisms of temperature perception are only emerging, plant hormones have been found as important factors modulating morphological responses to temperature changes. To provide an insight into the role of cytokinin in response to higher ambient temperatures, we employed the hypocotyl elongation assay and characterized in details temperature-dependent early development in a model plant *Arabidopsis thaliana*. Our results showed that conditional modulations of cytokinin levels or cytokinin signalling impair the growth response to higher ambient temperatures. In-depth analyses in transgenic plants and

mutants of morphological, hormonome, transcriptome and proteome responses revealed that increased temperature depletes cytokinin pool but sensitizes cytokinin signalling. We will present a number of important players in the temperature signalling and/or thermomorphogenesis, identified by the integrative approach.
This work was supported by *MEYS*, *project No. LQ1601* (CEITEC 2020) and *Czech Science Foundation grant P305/12/2144.*

O-78

Age-dependent modulation of hypoxia tolerance in Arabidopsis: a role for cytokinin?

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Oxygen concentrations in plant tissues can fall to very low levels, either due to high metabolic activity, or stresses such as flooding that restrict gas diffusion. Under low O_2 conditions (hypoxia) ATP production via aerobic respiration is inhibited, leading to an energy crisis and possibly death. Plant response to hypoxia is strongly influenced by developmental age. Genes involved in hypoxia acclimation are regulated differently in young seedlings compared to older seedlings or adult plants. However, it remains unclear in what way this is correlated to survival. Using the model species *Arabidopsis thaliana*, we discovered that seedlings of varying ages differ in hypoxia survival. A genome-wide transcriptome survey of 4 and 7-day old Arabidopsis seedling root tips revealed a possible role for cytokinin in the differential root tip survival in hypoxia. Preliminary results showed that exogenous addition of cytokinin increased hypoxia survival of root tips. We discuss the possible functional role of cytokinin in hypoxia survival with respect to effects on root meristem size, cell division rate and lateral root formation.

POSTERS
01. Biosynthesis and Metabolism

P-01-01
Cytokinin *N*-glucosylation restricts shoot apical meristem activity but is apparently dispensable for other major developmental processes in *Arabidopsis*

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Metabolic conjugation of cytokinins with sugars is considered as one of the major cytokinin inactivation pathways in different plant species. Two glycosyltransferases, UGT76C1 and UGT76C2, have been proposed to control the cytokinin *N*-glucosylation in Arabidopsis. High functional redundancy of the respective genes and their organization in a tandem gene array have hampered a comprehensive genetic analysis of this pathway. To overcome this obstacle, we employed the CRISPR/Cas9 system to generate several *ugt76c1 ugt76c2* double knockout lines. Measurements of endogenous cytokinin levels revealed that disruption of *UGT76C1* and *UGT76C2* leads to a complete loss of cytokinin *N*-glucosylation, confirming that the pathway is controlled exclusively by these two genes in Arabidopsis. Intriguingly, the lack of the cytokinin *N*-glucosylation had only subtle effects on the concentrations of other cytokinin metabolites and on cytokinin responses, suggesting robust feed-back mechanisms in cytokinin homeostasis. Nevertheless, detailed analysis of *ugt76c1 ugt76c2* double mutants revealed phenotypic changes indicative of an increased activity of the shoot apical meristem (SAM). The role of the cytokinin *N*-glucosylation pathway in modulating SAM function was supported by the analysis of a new *UGT76C2* promoter reporter construct, which was expressed in specific domains of the SAM.

P-01-02
Identification of putative IAA-amino acid conjugate hydrolases genes in barley

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Auxins represent a class of phytohormones essential for numerous processes throughout plant growth and development. Plants use several mechanisms to regulate level of the auxin indole-3-acetic acid (IAA), including the formation and hydrolysis of amide-linked conjugates that act

as storage or inactivation forms of the hormone. Our understanding of how level of this hormone is controlled remains still incomplete.
Hydrolysis of amide-linked conjugates is mediated by amidohydrolases through cleavage of the amide bond between the auxin and the conjugated amino acid. Mutant screens based on reduced sensitivity to biologically active IAA-amino acid in root growth inhibition assays in Arabidopsis plants led to a generation of a specific group of amidohydrolases called the ILR1-like (IAA-Leucine resistant- like; ILL). They are able to cleave IAA-amino acid conjugates *in vitro*. Perhaps they are allowing plants to utilize stored IAA when needed. The Arabidopsis plants defective in three of these amidohydrolases have phenotypes suggestive of low endogenous auxin levels, including shorter hypocotyls and fewer lateral roots and have higher levels of the IAA conjugates in compare to wild type.
In transcriptomic data from one day old barley (*Hordeum vulgare*, cultivar Golden promise) seedlings we were able to identified several upregulated genes (MLOC_14346, MLOC_43138, MLOC_51086 etc.), which possess probable IAA-amino acid hydrolase activity of proteins from iLR1-like family. Based on the sequences we also found out that they share the same domains and features as Zn peptidases and Zn-dependent exopeptidases, peptidase M20 family and amidohydrolases. By the use of RT- PCR we were able to identified expression profile of genes of our interest in different plant tissues (grain, spikes, roots, stems, leaf).
Our main ambition is to confirm the presence of IAA-amino acid conjugate hydrolases in barley, examine their function and elucidate their effect on crown-roots development.

P-01-03

Cytokinin *N*-glucosides: their involvement in the evolution of hormonal homeostatic mechanisms in plants and roles in control of plant development

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Glucosylation of cytokinins (CKs) at the *N*7- and *N*9-positions of the purine ring generally regarded as irreversible represents one of mechanisms controlling homeostasis of bioactive CKs in plants. The products of this metabolic pathway, CK-*N*7- and *N*9-glucosides, are known of widespread distribution in higher plant species, representing even the major CK metabolic forms in most of them. In our comprehensive screening, however, only very low levels or a total

absence of CK-*N*7- and *N*9-glucosides were found in fungal kingdom and non-vascular plants such as algae and mosses. These data together with predominance of *cis*-zeatin-type CKs in these species indicate a close interconnection between CK-*N*-glucosyltransferase pathway and formation of *cis*-zeatins in the evolutionary context.
The levels and quantitative ratios of CK-*N*7- and *N*9-glucosides differed during ontogenesis in selected plant species. Whereas CK-*N*7-glucosides were essentially inactive in the chlorophyll retention assay in detached oat (*Avena sativa* L. cv. Abel) leaf segments, CK-*N*9-glucosides exhibited relatively high antisenescent activity. In the same experimental system, the HPLC-MS analyses revealed considerably stronger metabolic conversions for *trans*-zeatin-*N*9-glucoside compared to the corresponding *N*7-glucoside, leading to the formation of *trans*-zeatin and some CK storage forms such as *trans*- and *cis*-zeatin-*O*-glucosides. These data argue against the general image of the CK-*N*-glucosides, at least those glycosylated at the *N*9-position, as biologically inactive and irreversible compounds. They indicate that CK-*N*9-glucosides are probably more relevant to CK biology than previously thought, being metabolized in plant cells in a distinct way compared to their CK-*N*7-counterparts and having some unique function(s) in plant tissues.

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P-01-04

Identification of new components and regulatory mechanisms of auxin metabolism in tobacco BY-2 cells

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In plant cells, Indole-3-acetic acid (IAA), the most common occurring auxin, is metabolized by several pathways. IAA metabolites are products of oxidation and/or conjugation. IAA is oxidized by recently identified DIOXYGENASE FOR AUXIN OXIDATION (DAO). Reversible conjugation involves amino acids, glucosyl or methyl groups catalyzed by GRETCHEN HAGEN 3 (GH3), glycosyltransferase (UGT) or methyltransferase (IAMT), respectively. However, the coordination of these pathways is complex and tissue-specific. Therefore, we aimed to uncover the regulatory mechanisms of auxin metabolism in simplified system of auxin-dependent tobacco cell line BY-2. By comparing metabolism of auxin in control cells and cells cultured in auxin-free medium, we show here that while 2,4-dichlorophenoxyacetic acid (2,4-D) metabolite profiles

from control and auxin-free cells revealed no difference, IAA metabolite profiles showed significant quantitative as well as qualitative changes. To link these changes to the expression levels of involved genes, we performed transcriptomic and proteomic analysis of control and auxin-free treated culture. Characterization of differentially expressed genes revealed novel regulatory relations including auxin-induced and auxin-repressed members within the same gene family, potential post-translational regulation of auxin oxidase and new candidate glycosyltransferase responsible for auxin conjugation. Constructs for inducible expressions, targeted knock out and GFP-fusion of selected genes in tobacco BY-2 cell culture were prepared to validate their role in the formation of particular IAA metabolite.
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P-01-05

Auxin homeostasis in endoplasmic reticulum

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Auxin (indole-3-acetic acid; IAA) plays an essential role in plant growth and development. Crucial for many developmental processes controlled by auxin is its spatial distribution within plant organs, tissues and individual cells. Each cell contains extensive set of various membrane-encased organelles, which together compose the endomembrane system. One type of endomembrane organelle - endoplasmic reticulum (ER), which is primarily responsible for protein synthesis, lipid anabolism and cell detoxification, has been recently shown to be involved in phytohormone signaling and metabolism. It's assumed that ER-localized auxin transporters (PIN5, PIN8 and PILS2, PILS5) contribute to the control of auxin subcellular homeostasis by regulating auxin fluxes between the cytosol and ER lumen. Moreover, auxin biosynthetic proteins from the YUCCA family (YUCCA4) can be also localized to ER membranes, hypothetically together with IAA-amido synthases and IAA-amidohydrolases (GH3 and ILR1, respectively). However, to obtain a complete understanding of ER-mediated regulation of auxin homeostasis on subcellular level, direct measurement of auxin metabolites in ER is highly needed.
Thus we performed analysis of auxin metabolome in ER isolated from suspension culture of *Arabidopsis thaliana*. ER-enriched fractions were prepared by optimized method based on density gradient ultracentrifugation and the purity was verified by immunoblotting. Free auxin, its major precursors and metabolites were analyzed by using LC-MS/MS. The procedure will be further employed in study of selected *Arabidopsis* ER auxin transport mutant cell lines. ER-specific analysis will provide novel information about compartmentation of auxin biosynthesis and metabolism within plant cell.

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P-01-06

Dispersive solid phase extraction as a new tool for plant hormone sample preparation

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Cytokinins and auxins are phytohormones controlling plant growth and development. Their analysis is difficult because of their presence in plants at very low concentrations (pmol/g fresh weight). Modern analytical procedures for the determination of cytokinins and auxin consist of sample pre-treatment and subsequent instrumental measurement of individual metabolites. Nowadays one of the most used analytical techniques is ultra-high performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). In general, the isolation of phytohormones involves multiple critical and often time-consuming steps based on extracting and purifying analytes from a complex plant matrix, which can cause serious problems in LC-MS/MS analysis. Therefore, the analysis of cytokinins and auxins employs the well-established solid phase extraction (SPE). However, several years ago the dispersive solid phase extraction (DSPE) has been introduced as an effective and robust method for purification of a wide range of analytes. The DSPE technique is based on the dispersion of solid phase particles in the liquid sample. We used DSPE as an alternative method for purification of the phytohormone groups. Our work was aimed to investigate the main parameters contributing to effectivity of purification in comparison with conventional SPE.

P-01-07

Profiling of plant hormones by utilization multi-immunoaffinity purification based on monoclonal antibodies

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Plant hormones are signal molecules that, like hormones in animals, have crucial roles in plant growth and development. However, their isolation and quantification are challenging because of their extremely low levels in plant tissues (pmol.g^{-1} fresh weight) and therefore require effective isolation and purification. We have developed specific multi-immunoaffinity method for the joint purification and isolation of cytokinins, auxins and abscisic acid together. This method is based on the preparation of a mixed immunoaffinity gel with specifically binding monoclonal antibodies again abscisic acid, indole-3(yl)-acetic acid and cytokinins. The properties and capacity of individual gels were tested with standards of plant hormones with different concentration levels. Thereafter, the individual gels were mixed in the ration 2:2:1 (antibodies again ABA:IAA:CKs) and tested of immunosorbent capacity and the effect of the complex plant matrix on the yield of the purification step. Multi-immunoaffinity purification was paired with a highly sensitive ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis. Concentration levels individual phytohormonal groups were determined in 10-days old plants of Arabidopsis thaliana. The levels of individual analytes (ABA, IAA, CKs) were quantified by comparing the ratio of endogenous hormones and internal standards of known concentration. The development of multi-immunoaffinity gel for selected phytohormones can greatly accelerate and simplify the process of purification from plant material by using one-step immunoaffinity purification.

P-01-08

Are cytokinin-*N7*- and *N9*-glucosides active players in cytokinin metabolisms?

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Cytokinins (CKs) represent a group of phytohormones involved in a wide variety of growth and developmental processes. One of important regulatory mechanisms controlling the complex process of CK homeostasis is their conjugation to a glucose molecule at *N*7- or *N*9-position. Since their discovery in plants, CK-*N*-glucosides have been largely overlooked being viewed as irreversible and inactive CK products. However, our recent findings indicated that *trans*-zeatin-

*N*9-glucoside (*tZ*9G) delays considerably chlorophyll degradation in oat (*Avena sativa* L. cv. Patrik) leaf segments compared to *trans*-zeatin-*N*7-glucoside (tZ7G). In the same experimental system, substantial amounts of *trans*-zeatin (*tZ*) formation after exogenous treatment with *tZ*9G was determined by HPLC-MS. In contrast, no activity was observed in samples incubated with N^6-(Δ^2-isopentenyl)adenine (iP) and its *N*7- and *N*9-glucosides indicating distinct metabolic regulation of *tZ*- and iP-type CKs in plant tissues.
In dicot species (tomato, Arabidopsis), a strong antisenescence activity was found for *tZ*7G as well. Additionaly, distinct effects of *tZ*7G and *tZ*9G on the root growth of maize and Arabidopsis were found. Whereas significantly shorter roots were recorded in maize plants cultured hydroponically in *tZ*9G compared to *tZ*7G solution, no changes of root length occured on MS agar plates containing *tZ*7G or *tZ*9G in Arabidopsis. The expression data from maize and Arabidopsis root growth assays revealed interesting interactions among CK-related genes in response to both *tZ*7G and *tZ*9G applications. Our findings suggest different action of CK-*N*7- and *N*9-glucosides in monocot and dicot species and substantially question the apparent and generally accepted biological inactivity and irreversibility of CK-*N*-glucoconjugates in plants.

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P-01-09

Canis familiaris tissues are characterized by different profiles of cytokinins typical of the tRNA degradation pathway

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Cytokinins (CKs) are a group of N6 modified adenine derivatives essential to plant growth and development. These phytohormones have also been documented in other organisms, including bacteria, insects and mammals. Currently, N6-isopentenyladenine (iP) is the only CK that hasbeen identified in mammals. In plant systems iP nucleotides (iPRP) are the first form of CK synthesized and act as a precursor to other CK types. To determine if a similar biosynthesis pathway may exist in mammals we invstigated the presence of 27 CKs in a wide selection of canine organs using high performace liquid chromotography-electrospray ionization-tandem mass spectrometry (HPLC-(+ESI)-MS/MS). Seven forms of CKs were detected in the majority of the analysed samples, including N^6-(Δ^2-isopentenyl) adenine-9-riboside (iPR), N^6-(Δ^2-isopentenyl) adenine-9-riboside-5'(either mono-, di- or tri-phosphate; iPRP), *cis*-zeatin-9-riboside (cisZR), *cis*-zeatin-9-riboside-5'(either mono-, di- or tri-phosphate; cisZRP), 2-methylthio-N^6-isopentenyladenine (2MeSiP), 2-methylthio-N^6-isopentenyladenosine (2MeSiPR)

and 2-methylthio-zeatin (2MeSZR). Total CK concentrations ranged from 1.96 pmol/gfwt (adrenal gland) to 1.40×10^3 pmol/gfwt (thyroid). This study provides evidence that mammals synthesize and process a diverse of CKs, includin cis- and 2MeS-type CKs.

P-01-10

Subcellular phytohormone profiling in *Arabidopsis* based on FAOS technique

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Plant hormones regulate most aspects of plant growth, development and ecological plasticity. Better knowledge of their intracellular distribution can shed new light on plant cell physiology and can help us to better understand homeostasis maintenance and spatial signaling on the organelles level. Phytohormone profiling at the subcellular levels depends on the effectiveness of used isolation methods. So far published protocols are mainly based on gradient (ultra)centrifugation. However, resolution power of these approaches may not be appropriate. Therefore, we are introducing Fluorescence-Assisted Organelle Sorting (**FAOS**), the innovative subcellular compartment separating technique based on general principles of flow cytometry. Combination of highly accurate FAOS with super sensitive mass spectrometry-based method provides unique approach for determination and quantification of organelle specific phytohormone profile. Our results demonstrate the potential to sort five different intact organelle populations according to compartment-specific fluorescence parameters. Moreover, monitoring of organelle condition changes during sorting process is one of the most important advantages of this introduced method. Data from LC-MS/MS analysis proves, that auxin and cytokinin profiles are niether significantly changed by sorting procedure nor by fluorescent dyes treatment application. For its high resolution and purity, we expect further utilizing FAOS technique not only for phytohormone profiling in selected organelles but also for other -omics approaches.

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P-01-11

Cytokinin biosynthesis and perception in poplar

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Cytokinins (CKs) play a part in many aspects of plant growth and development. They are adenine derivatives and occur naturally either with isoprenoid or aromatic side chain. We have utilized UHPLC-MS/MS to screen content of aromatic CKs in 13 *populus* species and selected *Populus* x *canadensis* (cv. *Robusta*) as a model for the presented work. Mass spectrometric data show transient increase in the endogenous levels of poplar aromatic CKs after daybreak while levels of tRNA derived *o*-topolin remain unchanged. To examine CK metabolism further, all nine isopentenyl transferase genes (*IPTs*) found in the poplar genome were cloned, sequenced and their expression in different tissues analyzed by qPCR. We have also managed to express eight out of nine *IPTs* in *Escherichia coli.* However, they aggregate in the form of inclusion bodies and it was not possible to obtain pure proteins for kinetic measurements. Six out of nine *IPT* genes were further subcloned into pMDC7 vector under estradiol inducible promoter for transformation of *Arabidopsis thaliana*. Gene expression, protein amounts and CK profiles are presented. All five CK receptors were cloned and binding affinity of coded proteins was studied in life-cell competitive receptor assay in *E. coli* with various isoprenoid and aromatic CKs. Specificity and functionality of all 5 poplar CK repceptors are presented.

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P-01-12

Cytokinin degradation in the endoplasmic reticulum: Molecular mechanisms and physiological relevance

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Cytokinin degradation is controlled by cytokinin oxidase/dehydrogenase (CKX) enzymes. The molecular and cellular behavior of these proteins is still not fully understood. Here we present that Arabidopsis CKX1 is a type-II single-pass membrane protein that predominantly localizes to the endoplasmic reticulum (ER), indicating that this CKX isoform is a genuine ER protein directly controlling the cytokinin concentrations in this cellular compartment. Interestingly, independent experiments revealed that CKX1 forms homodimers and homooligomers *in vivo*. The N terminus of CKX1 was necessary and sufficient for the protein oligomerization as well as for targeting and retention in the ER. We could show that protein-protein interaction is largely mediated by transmembrane helices and depends on a functional GxxxG-like interaction motif. Importantly, mutations preventing the CKX1 oligomerization interfere with its steady-state localization in the ER and impair the CKX1 biological activity by increasing its ER-associated degradation. Thus, the data provide evidence that oligomerization is an important mechanism regulating CKX1 biological activity and the cytokinin concentration in the ER. Presented work should contribute to the discussion about the functional relevance of the cytokinin pool in the ER and the cytokinin signaling from this compartment.

P-01-13

WUSCHEL **regulates auxin biosynthesis in stem cell niche to control the stem cell fate and organogenesis in Arabidopsis shoot apical meristems**

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Unlike animals, plants can form above ground organs throughout the life because of the continuous differentiation of stem cells in the shoot apical meristems (SAMs). The rate of stem cell self-renewal and differentiation is precisely controlled in the SAM by the regulatory networks. CLAVATA-WUSCHEL (CLV-WUS) feedback loop involving *CLV3*, *CLV1*,

and *WUS* explains how SAM retains the constant number of stem cells. Since maintenance of stem cell fate and their self-renewal is tightly linked with differentiation to maintain the growth in SAM. It is still unclear how stem cell promoting factors coordinate differentiation. In plants accumulation of auxin is a major prerequisite to trigger differentiation of organs at the flanks of the meristem. This is achieved by a combination of local biosynthesis and transport. We show here that *TRYPTOPHAN AMINOTRANSFERASE RELATED 2* (*TAR2*), a gene encoding key enzyme involved in auxin biosynthesis pathway, is negatively regulated by WUS in stem cell niche. *TAR2* expression is restricted to peripheral zone cells of the SAM. WUS directly binds to the cis-regulatory elements present within the *TAR2* promoter. We show by transient activation of *TAR2*that increase in auxin accumulation in SAM spurt organogenesis and untimely differentiation of stem cells leading to termination of SAM. WUS, in addition to, promoting stem cell fate in SAM, also regulates differentiation of stem cells by inhibiting auxin biosynthesis in the stem cell niche. Our findings provide evidence that local auxin biosynthesis plays a critical role in differentiation of stem cells in the SAM, and WUS directly regulates auxin biosynthesis in stem cell niche to control timely departure of stem cell daughters.

02. Signalling

P-02-01

Flavonoids enhance plant immunity via the accumulation of ROS and inhibition of auxin signaling in *Arabidopsis*

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Flavonoids are widely distributed low molecular weight secondary metabolites in plants. It has been known that flavonoids have roles in many facets of plant physiology, including nodulation, fertility, UV protection, allelopathy, flower coloring and defense. One of their interesting roles is the inhibition of auxin transport and induction of defense resistance. Here, we investigated that three flavonoids, quercetin, kaempferol and naringenin, function as activators to improve plant disease resistances.By DR5::GUS experiments we found that flavonoid represses auxin response. In addition, we found increased stability of Aux/IAA protein by the treatment of flavonoid, indicating that flavonoid inhibits auxin signaling in plants. The transcript levels ofPR1, SA biosynthesis genes have been investigated in response to flg22 treatment after pretreated flavonoid in Arabidopsis. As results, we found enhanced defense response in flavonoid-pretreated Arabidopsis against flg22. In gel kinase assay we showed that flavonoid activate MAP Kinases.These results suggest a possibility that flavonoids are components of plant immunity through the accumulation of ROS and SA, the inhibition of auxin signaling in Arabidopsis.

P-02-02

Investigating crosstalk between canonical and non-canonical auxin signalling pathways.

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Auxin controls virtually every aspect of plant development, and its polar transport is vital to allow normal patterning and morphogenesis within a variety of organs. In the canonical auxin signalling pathway, transcription factors known as auxin responsive factors (ARFs), are bound by AUX/IAA repressor proteins, via their C terminal PB1 domains in the absence of auxin. In the presence of auxin, AUX/IAA proteins are targeted for degradation, and ARFs form homodimers through their N-terminal DNA binding domains, modulating the activity of auxin responsive genes. In the *Arabidopsis thaliana* gynoecium an atypical ARF known as ETTIN (ETT/ARF3), which lacks the PB1 domain required to participate in canonical auxin signalling, interacts with other transcription factors in an auxin sensitive manner, to regulate patterning and morphogenesis. We hypothesise that crosstalk occurs between the canonical, and non-canonical ETT mediated signalling pathways, through the hetero-dimerization of typical ARFs with ETT, via the highly conserved ARF dimerization interface. Here we show evidence supporting ARF: ETT hetero-dimerization, and implying genetic crosstalk between two distinct auxin signalling pathways.

P-02-03

Diversification of cytokinin phosphotransfer signaling genes in *Medicago truncatula* and other legume genomes

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Legume plants grown on nitrogen-deprived soils can establish a symbiotic interaction with soil bacteria collectively referred to as Rhizobia. This interaction leads to the formation of root nodules, providing conditions favorable for atmospheric nitrogen fixation by the symbiotic bacteria, for the plant benefit. Nodule initiation results from a reprogramming of cortical cells that divide to form a primordium, and cytokinins (CK) are key regulators of this developmental process. CK signaling is based on a phosphorelay cascade, first described in *Arabidopsis thaliana*, termed the Two-Component System (TCS) cascade, involving successively Cytokinin-binding Histidine Kinase receptors (CHKs), phosphorelay proteins (HPT) shuttling between the

cytoplasm and nucleus, Type-B Response Regulators (RRBs) acting as transcription factors to activate the expression of CK primary response genes. The latter includes Type-A Response Regulators (RRAs), which are proposed to exert a negative feedback on CK signaling. To determine whether the nodulation capacity of legume plants is linked to a specific subset of TCS proteins, a genome-wide identification of TCS protein-encoding genes was performed in six legume genomes (*Cajanus cajan*; *Cicer arietinum*; *Glycine max*; *Phaseolus vulgaris*; *Lotus japonicus*; *Medicago truncatula*). A comparison with the *Vitis vinifera* and *A. thaliana* non legume plants revealed a striking expansion of non-canonical RRBs, leading to the emergence of either shorter RRBs or RRBs in legume genomes where the conserved phospho-accepting aspartate residue is most frequently replaced by a glutamate or an asparagine. Available *M. truncatula* genome-wide gene expression data covering different plant organs, including symbiotic root nodules, revealed the higher expression of a limited set of cytokinin-related TCS genes, namely MtCHK1/MtCRE1, MtHPT1, and MtRRB2 and MtRRB3, suggesting that they form a "core" module potentially acting in most organs including nodules.

P-02-04

CFB, a cytokinin-regulated gene encoding an F-box protein targeting CAS1, a key enzyme in plant sterol biosynthesis

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Protein degradation by the ubiquitin-26S proteasome pathway is important for the regulation of cellular processes, but the function of most F-box proteins relevant to substrate recognition is unknown. We describe the analysis of the gene Cytokinin-induced F-box encoding (*CFB*, AT3G44326), identified in a meta-analysis of cytokinin-related transcriptome studies as one of the most robust cytokinin response genes. F-box domain-dependent interaction with the E3 ubiquitin ligase complex component ASK1 classifies CFB as a functional F-box protein. Apart from F-box and transmembrane domains, CFB contains no known functional domains. *CFB* is expressed in all plant tissues, predominantly in root tissue. A *ProCFB:GFP-GUS* fusion gene showed strongest expression in the lateral root cap and during lateral root formation. CFB-GFP fusion proteins were mainly localized in the nucleus and the cytosol but also at the plasma membrane. *cfb* mutants had no discernible phenotype, but *CFB*overexpressing plants showed several defects, such as a white upper inflorescence stem, similar to the mutant line *cas1-1*, a hypomorphic mutant of the CAS1 gene encoding CYCLOARTENOL SYNTHASE 1, a key enzyme

in plant sterol biosynthesis. Both *CFB* overexpressing plants and *cas1-1* mutants accumulated the CAS1 substrate 2,3-oxidosqualene in the white stem tissue. Cytokinin treatment further increased the 2,3-oxidosqualene concentrations in the *cas1-1* mutants, corroborating the notion that CFB links cytokinin signaling with the sterol biosynthesis pathway in a negative regulatory manner.

P-02-05

Transcriptional analysis reveals key roles of sugars and cytokinins in triggering axillary bud outgrowth after decapitation

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The study of shoot branching is based on decapitation experiments where removing the shoot tip stimulates axillary bud outgrowth. However, the transcriptional activities and underlying signals triggering axillary bud outgrowth after decapitation are poorly understood. Using deep RNA sequencing (RNA-seq), we identified genes encoding the transcription factors, TEOSINTE BRANCHED, CYCLOIDEA and PROLIFERATING CELL FACTOR (TCP) 14 (TCP14) and SCARECROW-LIKE 4 (SCL4) to be significantly induced in axillary buds 1 hour after decapitation. Additionally, four sugar-starvation responsive (SSR) genes were significantly repressed in axillary buds 1 hour after decapitation. Using real-time quantitative PCR (qPCR), we found that genes rapidly induced in axillary buds after decapitation were also induced by treatments that stimulate shoot branching; sucrose feeding, cytokinin (CK) applications and repressed treatments that inhibit shoot branching; defoliation prior to decapitation and strigolactone (SL) applications. Genes rapidly repressed in axillary buds after decapitation were also repressed by sucrose feeding and CK applications and induced by defoliation prior to decapitation and SL applications. Finally, we show that ectopic and analogous overexpression of TCP14 and SCL4 respectively promotes shoot branching in Arabidopsis. Together, our data suggests that the induction of TCP14 and SCL4 and repression of SRR genes thereby integrating hormone and sugar signalling is involved in triggering axillary bud outgrowth after decapitation.

P-02-06

Molecular insights into auxin effect on PIN polarity

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The plant hormone auxin is needed for almost all aspect of plant life. Auxin has a unique ability to undergo a directional transport which can be modulated by auxin itself. The so-called canalization hypothesis propose feedback of auxin on its own cell-to-cell directional transport by controlling localization of PIN transporters in TIR1-dependent manner. The underlying mechanism of auxin effect on PIN polarity is largely unknown. In previous study, we used microarray approach to identify genes transcriptionally regulated by auxin which act downstream of SCF^{TIR1}-AUX/IAA-ARF auxin signaling. Besides the known molecular players involved in PIN polarity, WRKY23 transcription factor was identified and characterized as a novel regulator. Here, we followed another microarray approach to identify genes acting downstream of WRKY23 to execute the auxin effect on PIN polarity. We identified and verified role of several novel downstream regulators including a putative novel auxin importer and Receptor-like kinase.

P-02-07

Brassinosteroids mediated regulation of ABI3 is involved in high-temperature induced early flowering in plants

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ABSCISIC ACID-INSENSITIVE 3 (*ABI3*) is one of the essential transcription factors of ABSCISIC ACID (ABA) signaling, functioned in seed germination and abiotic stress tolerance. Recent study showed that epigenetic repression of *ABI3* by active BR signaling pathways is critical events for promoting seed germination and early seedling developments. However, other physiological roles of the BR-mediated regulation of *ABI3* and ABA responses are largely unknown. Here, we showed that BR signaling activation by high temperature promotes flowering time by suppression of *ABI3* expressions. Ectopic expression of *ABI3* specifically compromised early flowering phenotypes of *bes1-D*, and induced late flowering phenotypes in wild type. Both expression patterns and global transcriptome analysis supported the biological roles of *ABI3* in regulation of flowering time. Finally, we confirmed the lower expression of ABI3 by high-temperature induced BR signaling activity was correlated with early flowering phenotypes. In conclusion, our data suggest that BR-mediated regulation of ABI3 is importantly involved in high-temperature induced reproductive phase transition in plants.

P-02-08

Functional characterization of gibberellin receptors in *Panax ginseng*

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Gibberellins (GAs) are an essential phytohormone associated with diverse growth and developmental processes including cell elongation, seed germination and reproductive organ developments. Recent advances in genome sequencing technology and functional genomic analysis of model plants have provided significant information for GA signaling pathways and related genes. Although its economical and medicinal importance of *Panax ginseng* has long been considered, the functional genomic studies of GA signaling pathways in this crucial perennial herb plant have rarely carried out. We here conducted functional characterizations of eight *GID1*s in *P. ginseng* (*PgGID1A-H*). We confirmed that PgGID1s have evolutionally conserved important domains and residues for interaction with active GAs and DELLAs. Tissue specific expression patterns of *PgGID1s* during developmental processes of *P. ginseng* were explored with reanalysis of available RNA-seq data. Prediction and comparison of crystallographic structural similarity of PgGID1s with an AtGID1a supported their functions as a GA receptor. Also, the subcellular localization and GA-dependent interactions with the DELLA proteins of *P. ginseng* were similar to those of GIDs in other plants. Finally, we confirmed that overexpression of *PgGID1s* completely restored the GA-defect phenotypes of the *Atgid1a/c* double mutant. Thus, we reveal that PgGIDs function as GA receptors in *P. ginseng*. This critical information of *PgGID1*s has potential to be used genetic engineering of *P. ginseng* and to understand perennial plant developments.

P-02-09

A non-canonical auxin-signalling mechanism regulates gene expression by affecting chromatin state

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The phytohormone auxin acts as a key regulator of growth and development at every stage of a plant's life cycle. Canonical auxin signalling occurs through a pathway in which auxin molecules bind to TIR1/AFB F-box proteins, promoting their interaction with Aux/IAA repressor proteins and subsequently their degradation. This repressor degradation leads to de-repression of Auxin Response Factors (ARFs) and expression of ARF target genes. Recently, our group has found an alternative, non-canonical auxin signalling mechanism in which the atypical ARF, ETTIN (ETT/ARF3) physically interacts with diverse transcription factors (TFs) to regulate downstream targets in an auxin-sensitive manner. This mechanism is important for coordinated gynoecium development. Examining a range of chromatin marks associated with transcription activation and repression suggests auxin-dependent chromatin dynamics on ETT-target gene loci. In agreement with this, a Yeast-2-Hybrid screen revealed that several members of the TOPLESS/TOPLESS RELATED (TPL/TPR) family can interact with ETT in an auxin-sensitive manner through a conserved motif in its C-terminal domain. The TPL/TPR is a family of Groucho-Tup1-like co-repressors in plants that achieve transcriptional repression of gene expression through the recruitment of histone deacetylases. Previously, TPL/TPR proteins have been shown to interact physically with TFs including a number of ARFs and Aux/IAAs. The importance of TPL-Aux/IAA complexes in canonical auxin signalling is well-established while the

role of TPL-ARF complexes remains unknown. Here, we propose the existence of an auxin-signalling pathway in which ETT-TF complexes directly interact with co-repressors under low auxin conditions to repress expression of target genes. In contrast, under high auxin conditions, the interaction between ETT, TFs and co-repressors is modified to allow derepression of these targets.

P-02-10

Auxin rapid inhibition on root growth in *Arabidopsis*

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Phytohormone auxin promotes the hypocotyl growth, while inhibits the root growth in *Arabidopsis*. The Acid growth theory has been well verified in the hypocotyl. However, how auxin regulates cell expansion in the root is still largely unknown. Especially, auxin inhibits the root growth in such a rapid way that, on one hand, the timing of the effect of growth inhibition has been usually dismissed when other cellular processes are observed; on the other hand, the growth mechanism for such rapid response must be distinct from the counterpart in the hypocotyl.
Given that several mechanisms have been proposed as cell growth regulator in the root, such as vacuole fragmentation and microtubule reorientation. We take advantage of the microfluidic system, vacuole and microtubule marker lines, and mutants of vacuole and microtubule as well. We can visualize the exact timing of the change in root growth, cell length and markers, in order to reveal the sequence of the event in high time resolution and primarily sort out the causal relationship. In addition, we examine the acid growth theory in the root by manipulating the PM ATPase with Fusicoccin and overexpression of SAUR19, and we measure the apoplast pH via HPTS dye. Our data indicates that auxin increases pH during growth inhibition but surprisingly not via PM ATPase.

P-02-11

Bioactivity of N^6-benzyladenine derivatives assayed by interaction with the cytokinin receptors *in planta, in vitro,* and *in silico*

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Ligand preference of *Arabidopsis* cytokinin receptors AHK2, AHK3 and AHK4 was assayed *in planta*, by measuring activity of cytokinin-sensitive promoter, and *in vitro*, by determining ligand-receptor binding. Double receptor *Arabidopsis* mutants and 25 BA derivatives with various substituents in purine heterocycle, linker or aromatic moiety were used. Prominent correlations between ligand specificity profiles of receptors, particularly AHK2 and AHK4, corroborate the functional similarity of their hormone-binding sites. However, receptor properties are not identical, each receptor is distinctive in its ligand preference. Ability of ligand to bind to the receptor was highly correlated with intensity of the receptor signaling. Imperfect correlation testifies that high affinity binding of a ligand is a necessary but not sufficient condition for "switching on" the receptor. A few derivatives were found which activate preferably one of the receptors. These new receptor-specific cytokinins are promising for further studies. Receptor structure analysis, molecular modeling and molecular docking were employed to rationalize interaction patterns between ligands and individual receptors. The obtained dependencies between docking and experimental binding provide a basis for design of new receptor-specific cytokinin analogues.

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P-02-12

Cytokinin perception beyond flowering plants

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Cytokinin (CK) receptors were characterized in several flowering plants but little is known about properties of these proteins beyond Angiosperms. We analyzed ligand-binding properties of putative CK receptors from moss *Physcomitrella patens*, lycophyte *Selaginella moellendorffii* and spruce *Picea abies*. Transient expression of receptors was carried out in tobacco leaves from which microsomes were isolated for radioligand assays. Of the three known moss receptors, we succeeded to express and study PpCHK1 and PpCHK2. These receptors are clearly isopentenyladenine (iP)-type: *K*d of iP for PpCHK1 and PpCHK2 are as high as 1.8-1.9 nM. Affinity of *trans*-zeatin (tZ) for these proteins is much lower. In PpCHK3, an insert of 40 aa was

found in the sensory module inhibiting CK binding. Sensory modules of two putative receptors (SmCRE1-1 and SmCRE2-1) of the lycophyte were obtained by DNA synthesis. SmCRE1-1 was found to be functionally defective as it lacks an upstream transmembrane α-helix, a part of the sensory module and a conserved Asp residue critical for CK binding (replaced with Asn). Another receptor, SmCRE2-1, showed very low iP specific binding, close to background level. This receptor has an insertion of 4 amino acids followed by a deletion of 7 amino acids in conserved part of the sensory module. Thus, it is unclear how this plant achieves CK binding. In spruce genome, we found two putative receptor genes: *PaCHK1* and *PaCHK2*. We managed to clone and express the sensor module of PaCHK2. This receptor binds tZ and iP with high affinity preferring tZ (*K*d 1 nM). All investigated receptors have a pH-dependence of CK binding typical for intracellular proteins.

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P-02-13

Shaping signalling landscape during organ formation

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By forming lateral roots (LR), plants acquire anchorage and the ability to efficiently explore the soil for water and nutrients. Each phase of LR development is tightly regulated by dynamic and continuous cytokinin and auxin signalling. While the role of auxin is well understood, the knowledge on cytokinin production, distribution and perception in the context of LR formation is scarce. In addition, there is still a debate whether cytokinin perception occurs at the plasma membrane, endoplasmic reticulum, or both. Interestingly, several components of the cytokinin signalling pathway are misregulated in the spatial accommodating mutant *$CASP1_{pro}$::shy2-2* that is unable to make LRs. To get new insights into how and where cytokinin affects LR development, we will compare cytokinin signalling in this mutant and the wild type. Moreover, we will use cell type-specific manipulation of cytokinin synthesis, perception and signalling to reveal at subcellular resolution how extra- and intracellular cytokinin pools can affect patterning and formation of LRs. Furthermore, we will examine the role of PURINE PERMEASE (PUP) proteins, which shape the cytokinin signalling landscape by importing the hormone into the cell or endoplasmic reticulum before it can be perceived by the receptors. Preliminary results imply the involvement of PUP4, PUP14 and PUP18 in the LR development, and we will expand on this work by creating a root expression atlas of all members of the PUP family during LR formation. Together, these findings will help elucidate how cytokinin signalling operates in space and time to support the creation of a complex organ.

P-02-14

Diversity of auxin responsive cis-regulatory elements

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Phytohormone auxin regulates virtually every developmental process. Control of auxin responses is implemented via activation/inhibition of ARFs (Auxin Response Factors) transcription factors (TFs). ARFs known to bind TGTC-containing AuxRE (auxin-responsive cis-regulatory elements). However, the signalling beyond ARF-dependent response in largely unknown despite involvement of many other TFs was shown in many cases the mechanisms were not studied in detail.
We performed meta-analysis of all available auxin-induced transcriptome datasets to search for hexamers and bipartite sites enriched in promoters of auxin-responsive genes. Meta-analysis identified a comprehensive set of cis-regulatory elements associated with auxin up- and down-regulation in early or late response. Surprisingly, TGTC-containing elements were not the most highly overrepresented hexamers. Instead, bHLH- and bZIP- binding motives and a set of AT-rich motifs were the most strongly associated with auxin response. The lists of hexamers associated with up- and down-regulation in late response significantly overlap, though, the latter almost twice longer. For 28 genes driven by the regulatory regions with predicted bipartite elements auxin-inducibility was supported by RT-qPCR. Functionality of one predicted bipartite site was tested by mutagenesis. Though, a lot of unknown previously sites are left for further verification.

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P-02-15

Preparation and perception of fluorescently labeled isoprenoid cytokinins

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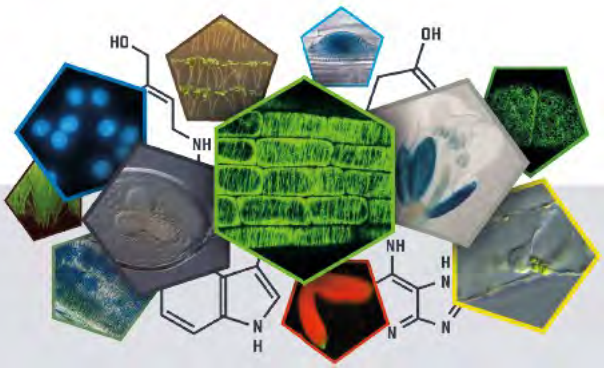
Isoprenoid cytokinins play crucial roles in the regulation of plant growth and development. To understand cytokinin receptors in plants, we designed and synthesized fluorescent derivatives of N^6-isopentenyladenine (iP) with several fluorescent labels attached to the C2 or N9 atom of the purine moiety *via* a 2- or 6-carbon linker. The fluorescent labels included dansyl, fluorescein, 7-nitrobenzofurazan, rhodamine B, coumarin, 7-(diethylamino)coumarin and cyanine 5 dye. All prepared compounds were screened for affinity to *Arabidopsis thaliana* cytokinin receptor (CRE1/AHK4). Although the iPs with attached fluorescent labels mostly lost their affinity to the receptor, several fluorescent derivatives retained decent binding properties. Three of these derivatives, two rhodamine B and one 4-chloro-7-nitrobenzofurazan labeled iP were tested for their interaction with CRE1/AHK4 and *Zea mays* cytokinin receptors in detail. We further revealed the ability of several derivatives to activate transcription of cytokinin response regulator *ARR5* in Arabidopsis seedlings. Selected rhodamine B C2-labeled isopentenyladenines with short and long linkers and 4-chloro-7-nitrobenzofurazan N9-labeled iP and their respective negative controls (fluorescently labeled 6-dimethylaminopurines) were used for *in planta* staining experiments in *Arabidopsis thaliana* cell suspension culture using live cell confocal microscopy. Clear intracellular signal distribution was observed, while negative fluorescent control used at the same concentration (5 μM) showed only weak cytoplasmic fluorescent signal.



P-02-16

Selective auxin agonists induce specific AUX/IAA protein degradation to modulate plant development

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Auxin phytohormones control most aspects of plant development through a complex and interconnected signaling network. In the presence of auxin, AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) transcriptional repressors are targeted for degradation by the SKP1-CULLIN1-F-BOX (SCF) ubiquitin-protein ligases containing TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX (TIR1/AFB). CULLIN1-neddylation is required for $SCF^{TIR1/AFB}$ functionality as exemplified by mutants deficient in the NEDD8-activating enzyme subunit AUXIN-RESISTANT 1 (AXR1). Here, we report a chemical biology screen that identifies four small molecules, RubNeddin1 to 4 (RN1 to 4), requiring AXR1 to modulate plant development. Among them, RN3 and RN4 trigger selective auxin responses at transcriptional, biochemical and morphological levels. This specific activity is explained by their ability to promote the interaction between TIR1 and a specific subset of AUX/IAA proteins and to stimulate the degradation of specific AUX/IAA combinations. Finally, via a genetic screen using RN4, we reveal that the chromatin remodeling ATPase BRAHMA is implicated in auxin-mediated apical hook development. These results demonstrate the potential of selective auxin agonists identified by this study to dissect auxin perception for different developmental functions.

P-02-17

Role of auxin response factor genes in stem cutting of poplar during adventitious root development. Digging into cambium transcriptional sequencing

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Adventitious root (AR) formation is a primary step in vegetative propagation. The successful adventitious rooting of hardwood stem cuttings is economically important for forest trees. The molecular mechanism of AR development in woody plants is poorly understood. This work

demonstrates that different kinds of phytohormone signalling, genes and transcription factors are involved in AR development in poplar genotypes. The role of the auxin transcription factors genes ARF6, ARF8 and ARF17 in adventitious rooting in poplar is demonstrated by overexpression or downregulations of these genes in transgenic poplar. Overexpression of PtARF6, PtARF8 or downregulation of PtARF17 significantly induced AR development while downregulation of PtARF6, PtARF8 reduced it. These results showed the direct involvement of PtARF6, PtARF8 and PtARF17 in AR development in poplar. The poplar genotype, OP42 has inherent ability to produce early and more number of AR as compared to the hybrid aspen clone T89 when grown in hydroponic condition. Taking advantage of this differential rooting behaviour, we analysed the cambium tissue specific genes expression using RNA-Seq. The Laser Capture Microdissection was used to dissect and collect the homogenous cambium tissue from stem cutting from both genotypes at time points T0 (immediately after stem cutting) and T1 (24-hour hydroponic condition). A total of 17, 997 genes were differentially expressed (at FDR ≤ 0.01 and LFC ≥ 0.5) in both the genotypes including time point T0 and T1. The differential gene expression, signalling pathways, GO, KEGG, were analysed, which provided clues to reveal the potential different types of molecular mechanism and signalling pathways involved in AR development in both T89 and OP42. Taken together, our results suggest that the PtARF6, PtARF8 and PtARF17 are likely involved in AR development though cooperating with a series of various other genes, which will be discussed.

P-02-18

A new class of compounds specifically induce adventitious roots in Arabidopsis hypocotyls

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In a small molecule screen we identified an inducer of root formation on intact and elongated hypocotyls of *Arabidopsis thaliana*. This molecule was named hysparin for hypocotyl specific adventitious root inducer. Hysparin displays a unique property that its root-inducing activity is exclusively harnessed via the cotyledons. In contrast to classic auxin-like molecules that cause retardation of primary root growth, hysparin does not affect the growth of the primary root. Here we report that hysparin does not augment classic auxin signalling yet its activity depends on part of the auxin signalling pathway. To investigate the selectivity of the compound, we performed a structure-activity relationship analysis with 35 hysparin analogues. We distinguished three classes of molecules: loss of root induction activity (18 compounds), typical synthetic auxin activity (11 compounds) or a hysparin-like activity (6

compounds). In addition to adventitious rooting we analysed primary root growth, lateral root induction, hypocotyl elongation and DR5-expression. From the analysis it follows that hysparin embodies a class of compounds that stimulate hypocotyl rooting and hypocotyl elongation distinct from classic auxins. Our results imply that strong accumulation of auxin is not an essential step in hypocotyl root induction. Further studies are directed towards the identification of Arabidopsis EMS mutants which are insensitive to hysparin, but show a normal response to synthetic auxins. This knowledge will help to unravel downstream signalling elements specifically required for hysparin-induced root formation.

P-02-19

Mechanisms promoting high-affinity interaction of auxin-responsive transcription factor with *cis*-regulatory elements

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Auxin triggers cell elongation and differentiation via proteasome-mediated derepression of the transcription factors, AUXIN RESPONSE FACTORs (ARFs). Several genes have been reported to act downstream of ARFs, and *in vitro* assays as well as promoter-reporter analyses have identified consensus DNA sequence motifs for auxin response, called auxin response elements (AuxREs). However, it is still obscure how specific interactions between ARFs and their target sites on genomic DNA are achieved.

We have been exploring the mechanistic framework for ARF-mediated transcriptional responses. Our previous structural and biochemical analyses revealed that ARFs can dimerize establishing strong binding affinity to AuxREs arranged in inverted repeat with a defined spacing. For *in planta* validation of the strong preference of ARFs for complex motifs, we analyzed transgenic Arabidopsis that carried fluorescent reporters driven by a promoter harboring inverted repeats of AuxREs. Furthermore, we assessed the biological impact of the palindromic AuxREs in effective gene activity, by mutant rescue assay focusing on vascular patterning. In addition, through meta-analysis of transcriptomic datasets obtained from auxin-treated samples, we examined the association of inverted and also direct repeats of AuxREs with auxin-responsiveness of genes. Together the results strongly support the topology-dependent cooperative action of AuxREs in ARF-DNA interaction and contribution of composite AuxREs to cellular auxin responses in plants.

P-02-20

Auxin and cytokinin-associated gene expression profile mediated by a redox active molecule nitric oxide in *Arabidopsis thaliana*

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Nitric oxide (NO), a highly reactive free radical, is an essential cellular regulatory molecule involved in a plethora of physiological processes in both animal and plant cells. As such, NO has been the center of attention in many fields of research. In plants, NO has a well-established role in key physiological processes relating to plant development and immunity. Increasing or decreasing NO levels can affect many important groups of genes involved in the major functional pathways of auxin and cytokinins. Functional annotation of our transcriptomic data identified GO terms involved in hormonal activity. A total of 199 differentially expressed genes (DEGs) involved in hormone metabolism were either up- or down-regulated as a result of S-nitrosocysteine (CySNO; NO donor) treatment. These DEGs were involved in biosynthesis, degradation, and signaling of phytohormones, such as auxin (IAA), cytokinin, gibberellic acid (GA), abscisic acid (ABA), and the defense hormones jasmonic acid (JA) and salicylic acid (SA). Trade-off between the Aux/IAA auxin-responsive transcriptional repressors and ARF transcriptional activators is important for normal auxin-dependent responses in Arabidopsis. The expression of transcriptional repressors Aux/IAA2-11 (AT5G43700), Aux/IAA14 (At4G14550), and Aux/IAA29 (AT4G32280) was reduced by 9, 11, and 28x, respectively, in response to treatment with 1 mM CySNO. Interestingly among cytokinin-associated gene family cytokinin oxidases (CKXs) which catalyze the degradation of cytokinins, only CKX4 was found to be significantly upregulated more than 40x in response to CysNO. This implies that as previously reported cytokinin can act as NO scavenger but it might be degraded by exogenous NO by significantly up-regulating CKX4 leading to the new insight for the role of NO in regulation Auxin and cytokinins.

P-02-21

New fluorescently labeled auxins display promising anti-auxin activity

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Plant hormone auxin influences all crucial stages of plant life cycle, thus numerous studies are devoted to unravel its specific role in individual physiological processes.
Fluorescent labeling of plant hormones allows sensitive and specific visualization of their subcellular localization and transport within plant tissues. Development of such methodological tools is of great importance for answering questions concerning plant growth and development. However, up to date only few fluorescently labeled auxins have been developed.
Herein, we report synthesis of novel fluorescently labeled auxin derivatives and validation of their biological activity and fluorescence properties. These compounds, unlike fluorescently labeled auxins published before, do not possess auxin activity but, on the contrary, inhibit auxin-induced effects, such as primary root growth inhibition, root hair growth and the auxin reporter *DR5::GUS* expression, in a dose-dependent manner. Moreover, we demonstrate that the length of the linker between the auxin and the fluorophore highly influences both the biological activity and the fluorescence properties of the compounds.

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03. Development

P-03-01

Characterization of adventitious root formation in Populus species and Norway spruce

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The root system of a plant is composed of the primary, lateral and adventitious roots (ARs). Adventitious roots form from stem or leaf derived cells. Adventitious rooting is an essential step in artificial vegetative propagation of plants. In horticulture, agriculture and forestry, vegetative or clonal propagation is widely used to multiply elite genotypes obtained in breeding programs or selected from natural populations. During the last decade, using the model plant *Arabidopsis thaliana* we showed that AR initiation is regulated by a transcriptional regulatory modules acting at the crosstalk of the auxin and jasmonate signaling pathways. Our aim is to take advantage of the knowledge acquired so far in Arabidopsis and go a step further in the understanding of key regulatory genetic factors controlling AR initiation in *P. trichocarpa* (easy-to-root) and *P. tremula x P. tremuloides* (difficult-to-root) species, by means of different approaches and we would like to adopt an evolutionary point of view and analyze how our knowledge translates to Norway spruce (*Picea abies*), which is the economically most important tree in Sweden. In this poster we will present the effect of different light regimes on adventitious root formation on hypocotyls of Norway spruce (*Picea abies*) de-rooted seedlings, and the consequences the different light conditions have on the hormone homeostasis. We also performed an anatomical characterisation of the different stages of development of AR in hypocotyls kept in different light conditions, which will be presented on this poster.

P-03-02

Can auxin-mediated competition fine-tuned by other players regulate pea axillary bud outgrowth?

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Shoot branching regulation, one of the fundamental developmental processes based on auxin and its interaction with other hormones, determines the overall architecture of aerial plant parts, but its exact mechanism has not been elucidated yet. Redirecting auxin flow to different points influenced which axillary bud formed the outgrowing and dominant shoot. Here we provide by auxin efflux inhibitor triiodobenzoic acid, proteosynthesis inhibitor cycloheximide as well as cytokinin and strigolactone treatment on a two-nodal-bud pea model system more insights into this phytohormone crosstalk during axillary bud outgrowth. Auxin canalization and its export into the main stem is crucial for regulation of bud outgrowth. Further, we show that cytokinins and strigolactone influence properties of auxin transport network.

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P-03-03

Organogenic activity of the *pin1* mutant inflorescence meristem.

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During organogenesis auxin is transported acropetally in the epidermis and L1 layer of the meristem. Concomitantly, auxin generates the maxima of its concentration in the organogenic zone, inducing the formation of the subsequent organs. In the developing primordia, auxin is then drained from the surface layer to the central tissues, towards the existing vascular system. In the inflorescence stem of *Arabidopsis thaliana* both the acropetal transport of auxin towards the organogenic zone and its subsequent basipetal drainage in the developing primordia are conditioned by the activity of the PIN1 proteins. In plants lacking the *PIN1* gene activity the acropetal transport of auxin to the meristem is blocked, causing the inhibition of organs initiation and the formation of a pin-like inflorescence stems. Interestingly, during further growth, despite inhibited polar auxin transport (PAT), some signs of the organogenic activity on the meristem start to appear. The aim of our project is to explain this phenomenon, and therefore we tested two following hypotheses: 1) in the *pin1* mutant, auxin is synthesized in the meristem at a certain stage of the inflorescence stem development; 2) auxin is transported to the meristem in a different to the PAT pathway. In both cases our assumption is that the emergence of the organogenic activity during later stages of the inflorescence development is an outcome of auxin accumulation in the meristem, due to blocked basipetal drainage by PIN1 proteins.

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P-03-04

Effect of exogenous auxins on somatic embryo formation and plant regeneration in spring barley anther culture in vitro

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Anthers culture in vitro is one of the most effective haploid biotechnologies allowing production of homozygous lines from hybrids of early generations (F_1–F_2). In this experimental system, microspore derived multicellular structures form, afterwards calli and somatic embryos emerge and plants regenerate under the influence of different factors. Among morphogenesis inducing

factors, composition of auxins and cytokins in nutrient media is considered to be the most important. The aim of our investigation was to determine an optimal combination of exogenous growth regulators for promotion of haploid production in spring barley anther culture in vitro. Anthers of DH-line with a high androgenetic capacity were inoculated on control inductive medium containing N6 macro-, MS micronutrients, organic supplements, 90 g/l maltose, 2 mg/l 2.4-D and 0.5 mg/l BAP. The experimental variants contained NAA (2 mg/l), FAA (2 mg/l, 10 mg/l), 2.4-D (0.5 mg/1, 10 mg/l). One media was hormone-free. Experimental results proved a possibility to regulate a mode of morphogenesis by application of certain growth regulators. Particularly, it was shown that application of 2 mg/l NAA instead of 2.4-D at the same concentration had a positive effect on direct somatic embryo formation. Replacement of 2.4-D with NAA resulted in an increase in morphogenic anther frequency from 44.9 to 53.1 % related to the total number of cultivated anthers. It is notable, that green plant regeneration frequency increased from 23.4 to 43.5 %. In addition, a significant advantage of NAA supplemented medium in comparison to that with 2.4-D lied in a significant decrease in the hyperhydrated plant frequency that resulted in a drastic rise in the plantlet survival rate. Hormone-free medium had no effect on the number of morphogenic anthers. However, this medium strongly inhibited plant regeneration due to its negative effect on embryo formation that resulted in intensive growth of calli with low morphogenic capacities.

P-03-05

Auxin and cytokinin signaling in the regulation of cambium activity in Arabidopsis root

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Auxin and cytokinin signaling pathways govern the primary patterning of root vascular tissues and also the activity of vascular cambium resulting in the formation of secondary xylem and phloem. In the primary vascular development, auxin and cytokinin signaling domains in the xylem axis and procambium, respectively, are largely non-overlapping. However, the hormonal signaling dynamics leading to the activation of secondary growth marked by periclinal (pro)cambial cell divisions are poorly known. Here, we show that procambial cell divisions can be prematurely triggered by cytokinin and auxin treatments, suggesting that sufficient hormonal signaling is required for secondary growth activation. During cambium activation, auxin and

cytokinin response markers are expressed in cambial cells, and cytokinin is shown to positively regulate auxin signaling. Furthermore, inhibition of cambium activation by the estradiol-inducible expression of *auxin resistant3-1* (*axr3-1*), a dominant negative regulator of auxin signaling, can be relieved by cytokinin treatment. Consistent with the phenotypes obtained during cambium activation, transcriptional profiling results show that expression of several cambial marker genes was decreased by *axr3-1* induction, increased by cytokinin treatment and intermediate expression was observed in the combined treatment. Our results also suggest that the symplastic connection between phloem and cambium is required for the hormonal regulation of secondary growth. In summary, we find auxin and cytokinin acting as positive regulators of root cambium activation in a synergistic manner.

P-03-06

Chemical screening reveals a role for ABA signalling in 2,4-D-induced somatic embryogenesis

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Somatic embryogenesis (SE) is a developmental process in which embryos are derived from vegetative cells of the plant rather than from fertilization. SE is generally induced by exposing explants to exogenous growth regulators, in particular auxinic herbicides. SE can be induced by 2,4-D in arabidopsis from different explants with varying efficiency. We used a chemical genomics approach to identify developmental pathways that enhance 2,4-D induced SE from mature arabidopsis seeds. Screening of the LATCA library identified 4-chloro-N-methyl-N-(2-methylphenyl) benzenesulfonamide (C1) as a powerful SE enhancer compound. The timing and location of somatic embryo formation did not appear to be different between C1 treated and control cultures. Moreover, microarray analysis showed that a short C1 treatment induced changes in gene expression that were also observed after 2,4-D treatment, including the expression of many genes involved in auxin- and seed specific ABA pathways. These data suggest that C1 and 2,4-D target the same developmental pathways. Genetic and molecular analysis showed that 2,4-D/2,4-D+C1-induced SE depends on primary ABA signalling, with major roles for the PYL10 ABA receptor and the ABI3 transcription factor.

P-03-07

The MADS-box transcription factor SEEDSTICK (STK) directly activates CKX7 controlling fruit elongation

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The fruit of *Arabidopsis thaliana* is dry and dehiscent with two lateral valves separated by the central replum that is directly in touch with the valve margin. So far, many transcription factors involved in the development of the fruit structure are already characterised, but it is still unknown the molecular mechanism that controls the correct elongation of the fruit and the final fruit length.

STK plays a crucial role in ovule development, and it is essential for seed release when the fruits are ripe. The *stk* mutant has shorter fruits compared to wild type.

Using the synthetic marker-line *TCS::GFP* we have noticed that *stk* mutant seems to have high cytokinin accumulation in fruit compared to wt. Furthermore ChIP experiments show that STK is able to bind the promoter sequence of CKX7 and it is able to activate it.

CKX7 is one of the seven members of the *CYTOKIN OXIDASE/DEHIDROGENASE* family of *A. thaliana*. This protein is the only CKX that acts in the cytosolic compartment of the cells.

The *ckx7* mutant has smaller fruits as *stk* mutant. These phenotypes suggesting that STK might controls fruit elongation, acting on the oxidation/degradation pathway of the cytosolic pool of cytokinins, through the regulation of *CKX7* expression.

P-03-08

Cytokinins control apical-basal developmental gradient in Arabidopsis via *NAC SECONDARY WALL THICKENING PROMOTING FACTORs*

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Mechanisms underlying formation of developmental gradients in plants remain elusive. We will present data showing that limiting endogenous phytohormones, cytokinins, or attenuated cytokinin signaling disturbs long-range developmental gradient in the inflorescence stem of Arabidopsis thaliana, leading to early secondary cell wall (SCW) formation in the vascular and interfascicular xylem. Using genome-wide transcriptional profiling we found that cytokinin (signaling) deficiency leads to the premature activation of a SCW transcriptional cascade controlled by NAC SECONDARY WALL THICKENING PROMOTING FACTORs (NSTs), the master regulators of SCW formation. In line with that, via generating triple and quadruple mutants, we provide genetic evidence suggesting that cytokinin signaling acts upstream of *NST1* and *NST3*. We identified expression gradient of *NST1* and *NST3* along the apical/basal axis of the inflorescence stem correlating with the developmental status of xylem cells and found out that the gradient is strongly disturbed in the cytokinin biosynthesis deficient lines. We show that cytokinins control the expression of *NST3* in a concentration-dependent manner and that exogenous cytokinin application is able to reconstitute apical/basal gradient of *NST3* as well as proper xylem development in the cytokinin biosynthesis deficient mutant. Both the cytokinin (signaling) deficiency and *NST* overexpression leads to the formation of tracheary elements (TEs) of smaller diameter, which consequently have strongly impaired hydraulic conductivity. We propose that cytokinins inhibit premature SCW formation in the apical part of the inflorescence stem by downregulating *NSTs*, facilitating thus the development of fully functional TEs.

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P-03-09

Regulation of fruit-shape formation in *Capsella rubella* reveals 'heart-breaking' details of hormonal and genetic interactions under tight control

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Fruit shape varies greatly between different genera of the Brassicaceae family. For example, while Arabidopsis fruits are cylindrical, fruits from the *Capsella* genus develop valves that are flattened and extended into pointy shoulders at the distal ends resulting in a heart-shaped appearance. Treatment with the auxin transport inhibitor, NPA, significantly reduce the shoulder formation, while two auxin biosynthesis genes were found to be specifically expressed in the shoulders along with the auxin-signalling reporter, *DR5::GUS*. Analysis of a PIN3-GFP reporter line suggests that auxin is transported from auxin maxima at the apex of the fruit valves towards the base. These data indicate an important role of auxin dynamics in regulating fruit growth and fruit shape in *Capsella*. To further elucidate the molecular mechanism involved in fruit-shape formation in *Capsella*, we carried out a forward mutagenesis screen for defects in fruit shape in the diploid *Capsella rubella*. One such mutant, called *heartbreak* (*htb*) produce fruits with reduced and deformed heart shape and a change of tissue polarity in the medial region. The underlying gene was cloned and found to encode a Small Ubiquitin-like Modifier (SUMO)-protease functioning in the SUMOylation pathway. Prior to fertilisation, the *HTB* gene is expressed throughout the gynoecium, but subsequently becomes restricted to the developing shoulders of the heart overlapping with *DR5::GUS* expression. Highly conserved potential SUMOylation sites in known key regulators of fruit development and their effect on auxin dynamics suggests the existence of a sophisticated network of hormonal and genetic activities fine-tuned by post-translational modifications to direct the formation of heart-shaped *Capsella* fruits.

P-03-10

Auxin and melatonin regulate the growth of wheat seedlings

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The question of whether the functions in the plants are identical for IAA and melatonin (Mel), which have tryptophan as a common precursor, remains open. The solution of the problem is

possible only after comparing the plant responses to the action of both hormones. The *Triticum aestivum* L. seedlings of Irgina variety were cultured on the media with IAA or Mel (0.1–100 μM) under white light (150 μmol quanta / m^2s). Analysis of the growth parameters of 8-day-old seedlings showed that the root extension depended on the concentrations of exogenous IAA or Mel. The high activity hormones were appeared in low concentrations (0.1 or 1 μM), under which the root length was increased by 10 and 16 %, respectively. At the same time, the highest IAA tested concentrations (10 or 100 μM) suppressed the root growth by 20 and 70 %, respectively. Mel in the same concentrations did not affect root extension. Previously, on wheat coleoptile segments, we showed that Mel increased efficiency of IAA treatment. By analogy with wheat coleoptiles, the effectiveness of Mel in the root could also be related with the endogenous IAA level. The more active growing coleoptiles was longer at 0.1–10 μM IAA and shorter at 100 μM. Exogenous IAA (0.1–10 μM) inhibited the stretching of the first leaf plate, whereas Mel did not affect the sheet size at the same concentrations. Stimulation of the coleoptile growth was determined by the inhibition of the first leaf growth due to redistribution of nutrients into the acceptor zone. The IAA inhibitory effect, which was typical for roots, was decreased in other part of the seedlings due to the buffer role of the root cortex in maintaining the homeostasis of the IAA endogenous level. Thus, the obtained data make it possible to say that the responses of wheat seedlings to the action of different concentrations of IAA and Mel are characterized by hormone- and organ-specificity.

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P-03-11

Interaction *AGAMOUS*-cytokinin in the control of floral meristem determinacy and gynoecium development in *Arabidopsis thaliana*

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Plants are able to produce organs and tissues during their life, this quality attributed to pluripotent stem cells located in specialized tissues called meristems. During the reproductive stage of *Arabidopsis,* the shoot apical meristem (SAM) located at the tip of the stem becomes the inflorescence shoot apical meristem (IM), which will generate floral meristems (FM) on its flanks. Subsequently, the floral organs are formed, whose identity is determined by the combination of transcription factors. The transcription factor *AGAMOUS* (*AG*) promotes the development of stamens and carpels. Furthermore, hormonal pathways are involved in signaling mechanisms related to flower development.
We want to understand how hormonal pathways such as cytokinin are integrated into local transcriptional networks and control specific organs like the gynoecium. In the current study, we hypothesize that, besides known genes identified during floral determination and gynoecium development, there are cytokinin-related targets of AG that play a role in early stages of gynoecium development. At the moment, we are focusing on the determination of the spatio-

temporal localization pattern of AG targets related with cytokinin signaling in early stages of flower development. Furthermore, we are analyzing the expression pattern of the synthetic cytokinin reporter *TCSn::GFP* as well as the synthetic auxin reporter *DR5::GFP* during early flower development. The latest results will be presented.

P-03-12

Physiological phenotyping of *Abies nordmanniana* as a basis for developing phytohormone-based strategies to improve Christmas tree production

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Abies nordmanniana is the major tree species cultivated for the production of Christmas trees and is therefore considered to be a high-value crop. The 12 years production time of *A. nordmanniana* trees is quite lengthy and the normal growth pattern results in tree morphologies that are not in favor of the consumers' wishes. During the first years, trees grow relatively compact with limited apical growth, while in later years the top leader elongation rate is too high. Understanding the physiological and metabolic mechanisms causing these growth patterns would allow for directed management to optimize production time and the product quality. In a physiological phenotyping approach, trees of distinctly different growth were analyzed for their phytohormone profiles comprising abscisic acid, auxin, various cytokinins, the ethylene precursor ACC, jasmonic acid, and salicylic acid, in tissue critical for tree growth and development. Profiling of these phytohormones was complemented by analysis of carbohydrate metabolism based on sugar content and central enzyme activities as well as by determination of antioxidative enzyme activities. The integrated data could be correlated with effects such as growth retardation of individual trees. These detailed analyses strongly contribute to our understanding of physiological and biochemical growth control in gymnosperm tree species. The knowledge gained from this physiological phenotyping approach will also directly feed into the development of phytohormone-based strategies to control growth and decrease cultivation time in Christmas tree production; concepts of these strategies will be discussed.

P-03-13

Auxin role in tissue pattern restoration after single-cell elimination in *Arabidopsis* root meristem

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Restoration of tissues following wounding is a physiologically crucial process in all multicellular organisms and in animals it largely relies on the targeted cell migration. Plant cells are enclosed by cell walls preventing their migration within tissues. Therefore, patterning in plant tissues relies largely on the regulation of cell divisions and the orientation of division planes in individual cells. We established single-cell laser ablation in the *Arabidopsis* root meristem, as an easy method to follow tissue-pattern disruption and restoration. We investigated roots using a vertical confocal microscope in real-time. Each elimination triggers a periclinal cell division specifically in the inner adjacent tissue. The outer daughter cell replaces the eliminated cell by gradually expanding in its direction and adopting its cell fate to the restore the tissue pattern. These observations raised multiple questions such as: How do the inner neighboring cells sense the adjacent cell death to become activated? How does the cell plane switches from anticlinal to periclinal? Which spatial signaling mechanisms convey the correct fate to the outer daughter cell?
We used marker lines for cell cycle progression, activation of periclinal cell divisions or wound signaling. This allowed us to observe how different treatments and genetic backgrounds affect the regulation of tissue pattern restoration. Disruption of auxin signaling highly disturbed the tightly controlled activation of division and inhibited a proper re-establishment of the original tissue pattern. We found that exogenously applied auxin acts as a constant mitogen in inner adjacent cells, and auxin biosynthesis is crucial for the enhanced progression of the cell cycle. Our work implies that auxin signaling tightly regulates the induction of mitosis and division plane reorientation after wounding, and is required for the correct restoration of the tissue pattern.

P-03-14

Kinetin derivatives with UVA and UVB photoprotective affect defend *Caenorhabditis elegans* against oxidative stress

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6-Furfurylaminopurine (Kinetin, Kin), has been described as a multiactive molecule with various activities both in animal and plant cells. New Kin derivatives were synthesized and screened for diverse biological activities. Standard routes of preparation via nucleophilic substitution of 6-chloropurine and 2,6-dichloropurine by appropriate amines were modified to obtain compounds in high purity and decent yields. Three standard cytokinin bioassays, tobacco callus, detached wheat leaf chlorophyll retention bioassay and *Amaranthus* bioassay were used to confirm cytoknin activity. Selected compounds were also tested on normal human dermal fibroblasts (NHDF) and keratinocyte cell lines (HaCaT) to exclude possible toxic and phototoxic effects. Furthermore, possible UVA and UVB photoprotective activity was studied. Naturally occurring antioxidant Rosmarinic acid was used as positive controls. Protection against oxidative stress showed by newly prepared cytokinin derivatives together with 6-furfurylamino-9-(tetrahydrofuran-2-yl)purine (Kin-THF) was further studied using induced oxidative stress (OS) on nematode *Caenorhabditis elegans* damaged by 5-hydroxy-1,4-naphthoquinone (juglone), a generator of reactive oxygen species. The most potent compounds were able to significantly protect human skin cells against UVA radiation *in vitro* and at the same time defend *C. elegans* against juglone induced OS *in vivo*. Finally, the ORAC assay showed that the compounds did not act as direct antioxidants as they were unable to directly scavenge oxygen radicals. These data suggest that the mechanism of protection against oxidative stress and UV irradiation is rather indirect and triggers other mechanisms than direct interaction with ROS.

P-03-15

Comparative analysis of plant DRL1/ELO4 and its yeast ortholog Kti12

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The Elongator complex was originally identified in yeast as a histone acetyltransferase complex that activates RNA polymerase II-mediated transcription. In a previous study, we showed that *DRL1/ELO4*gene, which encodes a homolog of the Elongator-associated protein Kti12 of yeast, acts as a positive regulator of shoot meristem activity and leaf polarity. Recent studies

reported that plant Elongator regulated auxin-related genes during transcription elongation. To determine the evolutionally conserved functions of DRL1 as an Elongator partner, we performed cross-complementation analysis between and DRL1 and Kti12.
Here, we show that the DRL1 motifs are conserved in yeast ortholog Kti12 and seem to be involved in binding of cofactors. Expression of the *DRL1* genes of plant in a yeast Kti2-deficient yeast mutant suppressed the growth retardation phenotype, but did not rescue the caffeine sensitivity, indicating that the role of plant DRL1 is partially conserved with yeast Kti12. Our results suggest the regions of species-specific sequence variation may thus provide first insights into the evolutionary differences between plant DRL1 and yeast Kti12. Taken together, we will discuss auxin responses in the establishment of leaf polarity by mediating Elongator complex.
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P-03-16

RNA methylation modulating cytokinin responsiveness

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Auxin and cytokinin antagonistically regulate plant vascular patterning in root. For example, auxin induces expression of *Arabidopsis Histidine phosphotransfer Protein 6* (*AHP6*) that inhibits cytokinin signalling in protoxylem while cytokinins up-regulates expression of PIN proteins in procambial cells resulting in efflux of auxin to xylem axis. This mutual inhibitory regulation of the two signals generates distinct domains of hormone responses to pattern vasculature in roots. In order to identify new regulatory factors in the networks, we carried out genetic screens focused on misexpression of *AHP6* in the background sensitized for cytokinin signalling (*cre1-12*). Consequently, we distinguished a mutant, *overachiever* (*ovac*), exhibiting expanded *AHP6* expression and reduced cytokinin responsiveness. Interestingly, the mutation can induce formation of a storage-root-like structure in *Arabidopsis thaliana*, characteristic to some crop species (such as radish or turnip) of the Brassicaceae family. Rough mapping and genome sequencing identified that the *ovac* mutant harbours premature stop codon in an uncharacterised RNA methyltransferase. We aim to characterise how the RNA methylation modulates cytokinin responsiveness and development in the Arabidopsis.

P-03-17

Association mapping of shoot-regenerative potential reveals natural variation in WUS and other hormone-mediated genes

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To identify which genetic elements underlie the capacity to regenerate shoots from root explants in *Arabidopsis thaliana*, a genome-wide association study was performed. Hereto, 150 natural accessions sequenced by the 1001 Genomes Consortium were subjected to a two-step regeneration protocol in which explants are first incubated on auxin-rich callus-inducing medium before being transferred to cytokinin-rich shoot induction medium. The results show that the regenerative potential of the strains is subject to considerable variation, with intermediate phenotypes indicating the involvement and interaction of multiple factors. Association with genome-wide sequence data reveals the importance of natural variation in the promoter of WUSCHEL, independent of modifications to the protocol. This is in line with recent reports that WUS is directly activated by CK-responsive ARRs to enable *de novo* specification of shoot meristems. Other significant loci include SNPs in or near auxin-response factors, MYB TFs, miRNAs, receptor-like kinases, F-box proteins and various biosynthetic enzymes. Many of these genes have not previously been related to organogenesis, but closer inspection unveiled homologues of *a priori* candidates such as WIND1, TIR1 and TSD2. Moreover, a large fraction of the proposed TFs and miRNAs interact with hormonal responses and regulate the expression of known players such as STM, KNATs and CUCs. Intriguingly, correlating the polymorphisms to 1001 transcriptomes uncovered that SNPs in UBC28 affect mRNA levels of this ubiquitin-conjugating enzyme. Apart from AT1G48820 (an unknown prenyltransferase), the latter also exhibits the strongest allelic distinction between poorly and well regenerating accessions. Validation efforts are now on the way, mainly using T-DNA lines. Other future goals include extension of the phenotypic data and performing one of the first methylome-wide association analyses in plants using WGBS data of over a 1000 strains.

P-03-18

Dissection of the polycomb response element of paternally imprinted *UPWARD CURLY LEAF1* during *Arabidopsis* endosperm development

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Genomic imprinting, an epigenetic process in mammals and flowering plants, refers to the differential expression of alleles of the same genes in a parent-of-origin-specific manner. Recent high-throughput sequencing analyses revealed that more than 200 loci are imprinted in *Arabidopsis*; however, only a few of these imprinted genes and their imprinting mechanisms have been examined in detail. Recently, we have reported that *UPWARD CURLY LEAF1* (*UCL1*), a gene encoding an E3 ligase that degrades the CURLY LEAF (CLF) polycomb protein, is a paternally expressed imprinted gene. After fertilization, paternally inherited *UCL1* is expressed in the endosperm, but not in the embryo. Polycomb Repressive Complex 2 (PRC2) silences the

maternal *UCL1* allele in the central cell prior to fertilization and in the endosperm after fertilization. In this report, we further analyzed the polycomb response element (PRE) of *UCL1* during endosperm development. A deletion between -2.5 and -2.4 kb upstream of the UCL1 translation start codon derepressed *UCL1::GUS* before fertilization, suggesting that PRE may be located in this region. PRE cooperated with the endosperm-specific factor binding element (ESFE) to drive the paternal imprinting and endosperm-specific expression of *UCL1::GUS*. PRE is active in a opposite orientation, although at a lower level. PRE required the DNA methylation region (DMR), located between –1.8 and –1.5 kb upstream of the UCL1 translation start codon, for maintenance of histone methylation for stable repression of the maternal *UCL1* allele. Thus, PRC2-mediated silencing of the maternal *UCL1* allele is regulated by both PRE and DMR, suggesting that divergent mechanisms for the regulation of PEGs evolved in *Arabidopsis*.

P-03-19

The role of CKX-interacting HIPP proteins in regulating plant development and cytokinin responses in Arabidopsis

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Maintenance of the optimal cytokinin concentration in plants requires a tightly coordinated regulation, for instance through the irreversible metabolic inactivation mediated by cytokinin oxidase/dehydrogenase (CKX) enzymes. In a yeast two-hybrid screen, we identified a distinct group of heavy metal-associated isoprenylated plant proteins (HIPPs) as CKX-interacting partners and confirmed the specific protein-protein complex formation by independent experiments. The HIPPs belong to a plant-specific metallochaperone-like protein family of 45 members in Arabidopsis characterized by the presence of a heavy metal-binding domain and a C-terminal posttranslational lipid modification. So far, HIPP proteins have been poorly studied and little is known about their biological function and molecular mode of action. The molecular and phenotypic analyses of several *hipp* loss-of-function mutants suggest that the identified *HIPP* genes are involved in various aspects of plant growth, such as root development and leaf formation. Experiments employing the synthetic cytokinin reporter *TCSn::GFP* in the *hipp* mutant background revealed changes in cytokinin status suggesting that the HIPP-CKX interaction is physiologically relevant for cytokinin activities *in planta*. The possible mechanisms underlying the HIPP-CKX interaction and the question how this interaction might modulate CKX activity and cytokinin responses are being currently investigated.

P-03-20

The role of auxin in baby-boom-mediated somatic embryogenesis

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Somatic embryogenesis is a form of induced plant cell totipotency where embryos derive from somatic or vegetative cells without fertilization. Somatic embryogenesis (SE) can be induced by exposure to stress or exogenous application of plant growth regulators, in particular the synthetic auxin 2,4-D. These SE induction treatments are thought to induce both stress response and endogenous auxin response. SE can be induced by ectopic expression of specific transcription factors, including BABY BOOM (BBM), an AINTEGUMENTA-LIKE AP2/ERF domain transcription factor. We used a combination of chemical biology and gene target identification to determine whether BBM also acts through auxin-related pathways during SE induction. Using ChIP-seq and mRNA-seq, we found that BBM bound and regulated the expression of auxin biosynthesis-, transport and signalling genes. BBM overexpression increased the level of indole acetic acid (IAA) at the beginning of SE induction, while chemical inhibition of auxin biosynthesis enzymatic activity abolished BBM-mediated somatic embryo formation. Blocking polar auxin transport with NPA also inhibited visible somatic embryo formation. Our data suggests that auxin-related events play important roles during the initial phase of BBM-mediated SE.

P-03-21

Investigating the role of the cuticle during apical hook development

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During skotomorphogenesis, the apical hook is transiently formed in order to protect the apical meristem from damage that could occur while the seedling is emerging from the soil. Apical hook development requires differential growth at the upper part of the hypocotyl and follows three consecutive phases: formation, maintenance and opening. Among the signals that regulate apical hook development, hormones such as auxin have been shown to play a major

role. In a search for new players in the modulation of hook development, we found that two cuticle-related mutants, *defective in cuticular ridges* (*dcr*) and *cytochrome p450, family 77, subfamily a, polypeptide 4* (*cyp77a4*), display impaired hook maintenance. *DCR* encodes an acyltransferase indispensable for normal cuticle formation and *CYP77A4* encodes an enzyme able to catalyze free fatty acid epoxidation *in vitro.* However, the mechanisms underlying the role of the cuticle in apical hook development remain unclear. Interestingly, the *dcr* mutant has been previously reported to be resistant to the inhibitory effect of cytokinin on etiolated hypocotyl elongation. In addition, the application of cytokinins promotes a sustained maintenance phase in wild type apical hook while the impaired hook maintenance in either *dcr* or *cyp77a4* can not be rescued by cytokinins. Further characterization of these cuticle-defective mutants will pave the way to a better understanding of the role of the cuticle and cytokinins in apical hook development.

P-03-22

The role of Purine Permeases in defining spatio-temporal cytokinin responses

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Recently, we have characterized the function of Arabidopsis Purine Permease 14 (PUP14) in confining the cytokinin responses. PUP14 is expressed complementary to the cytokinin responses throughout development. Its functional inactivation causes ectopic cytokinin signaling responses. PUP14 localizes to the plasma membranes and transports bioactive cytokinin to the cytoplasm, which represents a sink in cytokinin-perceiving cells. I will present further evidence to support this model, and show data about additional PUP members and their role in defining cytokinin signaling domains.

P-03-23

Dynamic response and functional significance of hormones during rice adventitious root development

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Phytohormones such as auxin and cytokinin are very critical endogenous regulators during plant development. Auxin is required in almost every aspect of root development in monocot and dicot plant species. The role of auxin is relatively well studied during root branching

in *Arabidopsis thaliana.* In general, its interaction with cytokinin regulates development of adventitious root (AR) and lateral root (LR) formation in *Arabidopsis*. However, cytokinin inhibits the initiation of LR primordia by preventing auxin gradient formation which is essentially required in the LR founder cells. Despite the key role of these hormones in adventitious root development, their responses have not been studied in rice. The sensitive synthetic reporter two-component signalling sensor (TCSn) is well reported in *Arabidopsis*and rice to monitor the cytokinin responses. We have generated transgenic rice lines harbouring such TCSn-GFP construct. We would present our analysis of spatio-temporal cytokinin responses at various stages of AR development through analysing GFP signals and transcript accumulation by RNA-RNA *in situ* hybridization. We would also present our data on the phenotypes observed upon removal of active pools of auxin in cytokinin responsive domains. Overall, we would provide insight on dynamic response of cytokinin and role of auxin in cytokinin responsive domain.

P-03-24

Auxin signaling repressor Aux/IAA12 is involved in root and leaf development in *Arabidopsis*

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Phytohormone auxin regulates almost every aspects of plant growth and development. Developmental responses to the plant hormone auxin are thought to be mediated by combinations of two interacting protein families: *Aux/IAAs*, short-lived transcriptional repressors, and ARF transcription factors. The stability of Aux/IAA proteins are regulated by $SCF^{TIR1/AFBs}$ type ubiquitin E3 ligase mediated polyubiqitination. The biological function of many Aux/IAAs were investigated by gain-of-function mutant studies. Previously, IAA12 was known to be involved in early stage of embryogenesis. However, the specific biological significance of IAA12 in other plant growth and development was not extensively studied yet. Therefore, in this study to investigate the biological function of IAA12, we constructed IAA12 overexpressing transgenic plant in Arabidopsis. IAA12 overexpressing transgenic plants causes short root hairs and reduced the number of root hairs as well as lightly down-curled leaves phenotypes compared to wild-type plant (Col-0). In addition, we found that stability of IAA12 was strongly enhanced by several environmental stresses. These results suggest that IAA12 plays a crucial role in root and leaf development and stress adaptive growth response.

P-03-25

Cytokinin signalling regulates organ identity via AHK4 receptor in *Arabidopsis*

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Plants are able to undergo postembryonic *de novo* organogenesis and thus effectively regenerate damaged tissues. Root or shoot identity of newly formed organs depends on mutual interactions of the phytohormones cytokinins and auxin. However, our understanding to the role of hormonal regulations in the cell fate reprograming is still elusive.
In the hypocotyl explant assay, auxin activated root formation while cytokinins mediated early loss of the root identity, disorganization of primordia structure and initiation of shoot development. Exogenous but also endogenous cytokinins influenced both the initiation of newly formed organs as well as the pace of organ developmental sequence. The process of *de novo* shoot apical meristem establishment was accompanied by strong activation of AHK4-mediated cytokinin signalling, induction of shoot-specific homeodomain regulator *WUSCHEL* specifically in the disorganized primordia and upregulation of endogenous isopentenyladenine-type cytokinins. Moreover, AHK4-controlled cytokinin signalling negatively regulates root stem cell organizer *WUSCHEL RELATED HOMEOBOX 5* in the root quiescent centre. We propose an important role of endogenous cytokinin biosynthesis and AHK4-mediated cytokinin signalling in the control of *de novo* induced organ identity.
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P-03-26

Cytokinin influences phytochrome-dependent seed germination in *Arabidopsis thaliana*

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Appropriate timing of seed germination is of crucial importance for a plant's life history. In *Arabidopsis thaliana*, seed germination is controlled by external cues, in particular light and water availability, but also by endogenous cues such as phytohormones. While the influence of ABA and GA in the regulation of germination is well documented, the functional role of cytokinin (CK) in germination has been investigated rarely. Here we show that *A. thaliana* seeds impaired

in CK biosynthesis, perception or signaling and seeds with an elevated CK catabolism have a dramatically increased light sensitivity of germination as compared to wild type, especially in the phytochrome A (phyA)-dependent 'very low fluence response' (VLFR). Interestingly, the increase in germination rates of seeds with a reduced CK perception was independent of changes in the absolute levels of ABA and the seeds´ ABA sensitivity. Furthermore, we could show that reduced CK perception of maternally derived seed tissue is particularly relevant to enhance germination under VLFR conditions. In a RNA-Seq analysis we identified, after the induction of germination with a light pulse, a large set of differentially regulated genes in CK receptor mutant seeds as compared to wild-type seeds. Gene expression data suggest that key light signaling genes are differentially regulated in CK receptor mutant seeds, including the gene encoding the far-red light sensor PHYA. In sum, in this work we discovered a novel function of CK in regulating seed germination with only very low amounts of light. This function will affect germination of seeds buried in the soil and consequently influence the population structure of *Arabidopsis* communities.

P-03-27

Root cap-derived cytokinin plays a role in determining root meristem size and lateral root initiation in *Arabidopsis*

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Lateral roots (LR) make a major contribution to the architecture of the root system and determine a substantial part of the ability of a plant to secure anchorage and extract micro- and macronutrients from the soil. The initiation and development of LR is regulated by plant hormones and environmental signals. Cytokinin (CK) is known to be a negative regulator of primary root growth and LR formation. Furthermore, CK acts as a positional cue for LR formation. Interestingly, the CK status is particularly high in the root cap (central columella and lateral root cap cells) but the functional relevance of this is unknown. Here, we aimed to investigate the eventual role of root cap CK in meristem size determination and LR pattern formation. To this end, we have generated transgenic plants that have a lowered CK content in the root cap and studied the consequences on root growth and development. The primary root meristem size of these transgenic plants was increased due to retarded cell differentiation. This was correlated with a decreased expression of *SHY2* and an increased expression of *DAR2* in transgenic plants compared to wild type. Further, transgenic plants displayed increased LR branching that was mainly attributed to an increase in LR priming/initiation events as marked

by *GATA23* expression. It has been shown before that root cap-derived auxin plays an important role in LR priming. We hypothesize that the reduced CK status in root cap cells perturbs the auxin status and transport thereby regulating the meristem size and also LR priming.

P-03-28

Investigating the role of cytokinin-inducible *EXPANSINs* in the control of cell wall properties and development in *Arabidopsis.*

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Plant cell walls (CWs) have the extraordinary property of combining extreme tensile strength with extensibility and studies of growth regulation suggests that turgor-driven cell expansion is the result of a delicate balance between wall relaxation and stiffening linked by a mechanosensing feedback loop. These regulatory networks, comprising hormones and transcription factors, regulate the collective behaviour of cell growth within a tissue. Phytohormones including cytokinins (CKs), are key players in growth regulation responses and are thus determinants of plant architecture and CW development. Our recent preliminary data suggest an important role for CK-regulated genes, namely *EXPANSINs*, in the control of CW properties. We plan to investigate the novel role for CKs in the regulation of CW composition and structure during CK-controlled cell differentiation. We hypothesize that CKs control cell differentiation via regulation of biomechanical properties of the CW.
We have identified a subset of CK-inducible members of the expansin family in *A. thaliana* and generated their translational fusions with a red fluorescent protein. We want to develop a biomechanical sensing model that describes the relationship between CK-controlled expansins and the mechanical properties of the CW during cell differentiation at the root-apical meristem. Our model will also include detailed characterization of spatio-temporal specificity of CK-controlled *AtEXPA* expression. We would like to use fluorescence emission-Brillouin scattering imaging for parallel measurements of mechanical properties and fluorescence in living cells.
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P-03-29

Nodules and lateral roots share developmental programs during initiation

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Plant growth and development are dependent on the macronutrient nitrogen. To overcome nitrogen deficiencies, legumes have acquired the ability to enter symbioses with nitrogen fixing rhizobial bacteria. To host these beneficial bacteria, legumes develop lateral root organs called nodules that develop at the differentiation zone of roots in response to successful infection with symbiotic nitrogen fixing rhizobial bacteria. It has been shown that the cytokinin signalling component *CYTOKININ RESPONSE 1 (CRE1)* and the upregulation of the transcriptional regulator *NODULE INCEPTION* (*NIN*) play a crucial role during nodule initiation. Furthermore, it has been suggested that cytokinin signalling perturbs the root-ward polar auxin flux in the stele at the site of infection and generates an auxin maxima sufficient to initiate pericycle cell divisions. To better understand the hormone dynamics and regulators required for nodule initiation we undertook expression analyses with high spatial and temporal resolution on rhizobial inoculated wild-type, *cre1*, and *nin* root sections combined with deep tissue imaging during nodule initiation. Our work provides evidence that *NIN* functions as a component of cytokinin signalling and is necessary and sufficient for induction of two *YUCCA* genes, which catalyse local auxin biosynthesis. Our work implies that the auxin maximum observed during nodule organogenesis is a function of the localised induction of auxin biosynthesis activated by cytokinin signalling. Downstream of this local auxin accumulation we found several transcriptional regulators that are also required for cell cycle activation during lateral root prepatterning and initiation, for example *LATERAL ORGAN BOUNDARIES DOMAIN 16 (LBD16)* which is required for lateral root and nodule development. Our data suggests that nodules and lateral roots share a similar initiation program and appear to diverge in shape and function during subsequent primordium development.

P-03-30

Thidiazuron Improves Shoot Regeneration of *Campanula* Species

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Ornamental *Campanula* species are cultivated as potted plants, cut flowers and garden plants. Especially in Europe the popularity of *Campanula* is of great importance. In order to maintain a competitive position among other popular ornamental species an intensive breeding program is necessary to insure the improvement of quality of produced plants. One of the methods for such improvements is genetic modification, for which efficient regeneration protocols are necessary.
The studies for establishment of successful regeneration system in two species *Campanula carpatica* and *Campanula portenschlagiana* have been performed. A range of cytokinins in combination with auxins were used. The best results were achieved by using Thidiazuron (TDZ), which was much more effective for induction of adventitious shoot formation than treatments with other cytokinins.

P-03-31

Identification of novel transcriptional regulators of local auxin biosynthesis during embryo and fruit morphogenesis in *Arabidopsis thaliana*

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The plant growth hormone auxin is instrumental for the establishment of plant body axes and organ patterning. Its action relies on its dynamic asymmetric distribution formed as the result of combination of local auxin production, auxin homeostasis and cell-to-cell polar auxin transport. Although the understanding of the auxin transport and its role in auxin responsive gene regulation has greatly advanced, the spatial and temporal regulation of local auxin biosynthesis is still poorly understood. The identification of local auxin biosynthesis enyzmes encoded by *TAA1* and *YUCCA* genes, showed that the local auxin biosynthesis plays a key role in major developmental processes including embryo and fruit morphogenesis. In our attempt of finding novel transcriptional factors (TFs) that control the specific spatio-temporal pattern of *TAA1/YUC* expression in *Arabidopsis thaliana* embryo and fruit development, we employed the yeast one-hybrid screen on *TAA1* and *YUC* promoter fragments. We identified several potentially novel transcriptional regulators of auxin biosynthesis, among which members of the TF families known to play key roles in the development of female reproductive organs, establishment of floral asymmetry, lateral organ developmet, seed germination and gametophyte development. As some of the identified TFs are known to form functional complexes with the members of the same and other TF familes, we believe to have possibly found few novel regulatory circuits of auxin biosynthetic genes in Arabidopsis fruit and embryo development.

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P-03-32

Multifaceted activity of cytokinin in leaf development shapes its size and structure in Arabidopsis

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At the cellular level, final size and structure of a leaf results from precise control of two fundamental processes – cell proliferation and expansion. Cytokinin (CK) has been shown to affect many aspects of leave development ranging from regulation of apical meristem to leaf senescence. To get a deeper insight into CK functions in distinct phases of leave development, we have employed a system enabling up- and/or down-regulation of CK content at distinct phases of leaf development. We report that increased CK content during the cell proliferation stage prolongs cell proliferation, and inhibits cell expansion and the onset of photosynthesis which is in line with their tight coupling. Down-regulation of CK content results in suppression of cell division and premature onset of cell expansion which partly compensates for decreased cell number. In contrast, during the cell expansion phase, CK regulates positively cell expansion and negatively cell proliferation as evidenced by reduced meristemoid divisions and a consequent reduction in the stomatal index. Transcriptome and proteome profiling revealed that the CK action is largely mediated by regulation of other growth rather than cell cycle regulators. Further, CK may support cell elongation via its positive effect on cell metabolism. Taken together, our data reveal a dual role of CK in the regulation of cell proliferation and elongation in distinct phases of leaf development.

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P-03-33

Auxin response in the desmidian alga *Closterium*

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Land plants have been preceded in evolution by the freshwater algae called 'charophytes'. Important insight into plant evolution is being gained by obtaining sequence data. However, to understand the basal evolution of many regulatory pathways, e.g. phytohormone-related, it is necesarry to establish these organisms in molecular plant biology. *Closterium peracerosum-strigosum-littorale* complex belongs to the desmids, single-celled algae phylogenetically close to land plants, with available gene transformation protocols. It is therefore an ideal candidate to start experimental research in charophytes. We found that *Closterium* shows a dramatic response to exogenously applied auxin, indole-3-acetic acid (IAA), which is known for its morphogenic role in the development of comparatively very complex bodies of land plants. When cultivated in medium enriched with micromolar concentrations of IAA, the algal culture showed inhibited growth with subsequent cell death. Moreover, in the later stages of subculture interval, mis-shaped cells were frequently observed, suggesting the effect of auxin on cell morphogenesis. Effort is currently underway to investigate the native metabolic profile of auxin in *Closterium*, as well as the optimization of methods for gene transformation, over-expression and knockouts preparation, in the aim to study the localization and function of *Closterium* homologs of auxin transporter genes.
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P-03-34

Light quality affects auxin and cytokinin responses in meristematic regions.

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For horticultural crops, such as tomato, the initial growth phase is crucial for the success of the whole production cycle. Growing young plants in multilayer climate chambers under LED lighting does not only provide an energy efficient solution, but also allows to steer plant growth by optimizing the spectrum, intensity and direction of light. However, this can only be realized if we have a profound understanding of regulation of growth and development by light. The main

objective of this project is to investigate how light signaling affects leaf initiation and root development during early development of tomato and *Arabidopsis* plants. In particular, we aim to understand how this is modulated by signaling of the key phytohormones auxin and cytokinin within meristematic regions. As a first approach, we investigated the effects of light quality on signaling of these hormones inside the root meristem, and how this affects root growth and development. Additionally, we compared photoreceptor mutants to wildtype *Arabidopsis* seedlings. Finally, since grafting is a general procedure in the production of young tomato plants, we also studied how light quality affects auxin and cytokinin responses during the post-grafting recovery of seedlings.

P-03-35

Strigolactones: from tree architecture to wood formation

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Trees play important roles in moderating the climate and providing habitat and biomass. So understanding the molecular mechanisms controlling the tree development, reproduction, architecture and wood formation become the base for modification of wood quality and tree breeding according environmental and economic needs.
Because trees have long generation time, a model for forward genetics so far remained elusive. Here we propose a new model tree, silver birch (*Betula pendula*). Comparing to other tree species, birch has shorter generation time (9 months) in flowering-induction condition, and birch has smaller genome (435 Mbp). Moreover, birch is monoecious, which is easier to out-crossed or inbred. These advantages facilitate tree research through multiple approaches.
The current focus of my study is on a naturally occurring silver birch variant, *Betula pendula var. "Kanttarelli"*, which has an intriguing bush-like phenotype. Through genome wide sequencing and candidate gene approach we found a point mutation resulting in an early stop codon in *BpMAX1* gene of the "*Kanttarelli"* tree. This gene is the only birch ortholog of Arabidopsis *AtMAX1* gene, which encodes a major strigolactones (SLs) biosynthesis enzyme. SLs are important regulators of plant branching: in Arabidopsis, *max1* loss-of-function mutant displays excessive branching and reduced apical dominance. As SLs is a graft-transmissible signal, we performed the grafting and the "*Kanttarelli*" phenotype was rescued by grafting. Combine the sequencing and grafting results together, we consider "*Kanttarelli*" is a strigolactones-deficient mutant.

In order to understand how SLs affect tree architecture and wood formation, RNAi and CRISPR (against *BpMAX1*) lines has been generated. The transgenic lines will be compared and analyzed for both tree architecture and wood formation studies. As expected, the RNAi callus was more bushy then the wild type birch during the agrobacterium-mediated transformation process.

P-03-36

Control of Stem cell division by fine-tuning Cyclin-dependent kinase activity

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Precise control of stem cell division is crucial for continuous organ formation and plant growth. Stem cells undergo two kinds of cell division, symmetric and asymmetirc cell division. Symmetric cell division, in which daughter cells have stem cell fate, increases the number of stem cells. On the other hand, during asymmetric cell division, one daughter cell has stem cell fate and the other starts to differentiate thereby forming organs. However, the mechanism of asymmetric division of stem cells is largely unknown in plants.
Previous studies showed that **CYCLIN-DEPENDENT KINASE (CDK)**, a master regulator of the cell cycle, is essential not only for cell division but also for the control of the stem cell differentiation. When both daughter cells after stem cell division have a high **CDK** activity, they maintain the stem cell fate. In contrast, when one of two daughter cell has a reduced level of **CDK** activity, it undergoes asymmetric cell division to produce a differentiated cell. However, **CDKs** are ubiquitously expressed in the root tip, indicating that unknown factors regulate the **CDK** activity and fine-tune the stem cell number.
Here we focus on **CDK INHIBITORs (CKIs)** which binds cyclin-CDK and inhibit the kinase activity. Phenotypic and expression analysis showed that **CKIs** play an important role in asymmetric division of stem cells in *Arabidopsis.*

P-03-37

Broad spectrum developmental role of Brachypodium AUX1

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Targeted cellular distribution of auxin is essential for various developmental processes. In *Arabidopsis thaliana* (Arabidopsis), amongst others, auxin influx carrier AUX1 and its LIKE AUX1 (LAX) homologs are important in this distribution. *AUX1* and its homologs are not strictly essential for the Arabidopsis life cycle. *aux1 lax1 lax2 lax3* quadruple knock outs are mostly viable and fertile, and strong phenotypes are not often observed.
In contrast, our research shows that the *Brachypodium distachyon* (Brachypodium) AUX1 homolog *BdAUX1* is essential for Brachypodium development. *Bdaux1* loss-of-function mutants are infertile dwarfs. Mutant roots are agravitropic like in Arabidopsis, however they are also longer and thinner than wild type roots because of increased cell elongation. Unexpectedly, higher free auxin levels were found in *Bdaux1* roots. Phenotypes and transcriptome data resemble those of other mutants in Brachypodium that have an increased amount of auxin.
Overall our results imply different wiring of auxin import in Brachypodium roots versus Arabidopsis. They reveal an essential role of *BdAUX1* in several developmental processes and suggest a more central role for AUX1 in pooideae.

P-03-38

Auxin impacts cell wall integrity by regulating xyloglucan composition in *Arabidopsis thaliana* hypocotyls

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Cell walls are composed mainly of cellulose, hemicellulose (xyloglucans and arabinoxylans), pectins and proteins. They provide rigidity and shape to plant cells, and in order to accommodate growth, plants need mechanisms to loosen, and then rigidify them. Auxin is crucial for growth regulation, both promoting and repressing it, depending on its concentration and the underlying cell type. However, the mechanism by which auxin modulates the cell wall is still largely elusive. We revealed that the PILS5-dependent downregulation of auxin signaling correlates with downregulation of several genes encoding for xyloglucan (XyG) modifying enzymes. In agreement, the overexpression of PILS5 induced changes in XyG composition, including an increase in fucosylation. Notably, the constitutive expression of a fucosidase did abolish PILS5-dependent repression of dark grown hypocotyl growth. Based on this data, it is possible to speculate of a role of auxin in regulating XyG composition for growth control. In agreement, we isolated mutants with specific defects in XyG composition showing hypersensitivity to exogenously applied auxin and enhanced auxin-dependent gravitropic response. These findings suggest that auxin affects cell wall integrity in part by controlling xyloglucan composition, which in turn contributes to auxin-dependent growth regulation.

P-03-39

Lush Spike – understanding the role of phytohormones during spikelet survival in barley

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Improving grain yield is a major objective of crop breeding, and a promising avenue for maximizing yield is through enhanced spikelet survival during pre-anthesis development. However, little is known about spikelet survival and its impact on grain yield in cereals. In our ERC-funded project titled "Lush spike," we are attempting to unravel the genetics and mechanism of spikelet survival in barley. In the genetics part of the study, we aim to discover QTLs for spikelet survival in a GWAS panel and validate them in bi-parental doubled-haploid (DH) mapping populations. Furthermore, interesting QTLs will be mendelized, functionally characterized, and the underlying gene will be identified using a map-based approach. To understand the mechanism of spikelet survival, we performed a histological study during spikelet development and growth in which it was found that pattern of spikelet development and growth followed by abortion resembles leaf growth and senescence. The vast available information on the influence of various phytohormones on leaf growth and senescence leads to study the distribution and quantification of phytohormones during spikelet development and growth. Additionally, the histologic and hormone studies will be complemented with the tissue-specific transcriptome and metabolome analysis. Finally, the spatio-temporal patterns of transcript, metabolite and phytohormone distribution/modulation in the spike may illustrate the mechanistical regulation of spikelet survival.

P-03-40

Hormone regulation of leaf morphology in rice

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Leaves are initiated from the flank of shoot apical meristem. Leaf development usually have three axis: distal-proximal; adxial-abxial and medial-lateral. Screening of rice mutants for different leaf morphology results in several leaf defect mutants. We focused on two mutants, one has narrow leaf (*nl1*) and another one has wider leaf (*wl1*) compared to wild type. In addition, we found that the primary root length is decreased in *nl1* while increased in *wl1.* The *NL1* and *WL1* were mapped to chromosome 1 and 3, respectively. *NL1* encodes an auxin biosynthesis gene which is a homolog of *TAA1* gene in Arabidopsis. We found a C to T mutation in the *nl1* mutant, which cause the premature termination of OsTAA1 and resulted in the deficient of auxin biosynthesis. *WL1* encodes an zinc finger transcription factor which regulate the expression of *OsCKXs* thus modulate cytokinin metabolism. We found a 18bp deletion of *WL1* in *wl1* mutant which may resulted in the suppression of *OsCKXs* and the increase of cytokinin content. Cross-section through mature leaf blade exhibited that *nl1* mutants developed less vascular bundle compared to wild type. In

contrast, *wl1* mutants have more leaf vascular bundles. These results suggest that auxin and cytokinin are involved in vascular bundles development in rice which play important role in final leaf morphology determination.

P-03-41

Auxin is a main hormonal factor of petunia pollen tube growth

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A fine-tuned network of cellular processes is required to regulate the germination of pollen grains and the elongation of pollen tubes. To elucidate whether plant hormones are involved in these processes, the effects of exogenous phytohormones, indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellin A3 (GA3) and cytokinin (kinetin) on the growth, PM polarization, actin cytoskeleton organization and cytoplasmic pH (pHc) of in vitro 4 h-growing petunia pollen tubes were investigated. IAA, ABA and GA3 displayed the growth-stimulating effects and these were accompanied by orthovanadate-sensitive hyperpolarization of the PM. Kinetin has no effect on the membrane potential of pollen tubes. Fluorescent labeling the enzyme with H+-ATPase antibodies exhibited IAA- and ABA-induced lateral PM redistribution into the subapical zone of pollen tube PM. Pollen cultivation on the medium with latrunculin B, the inhibitor of actin polymerization, resulted in inhibition of tube growth and simultaneously in the drop of endogenous IAA content. The IAA-growth stimulating effect was correlated with increased content of actin filaments in both apical and subapical zones of tubes. In contrast, kinetin decreased the total F-actin content. In the case of male gametophyte growing for 1, 2 and 4 h, IAA induced alkalinization of the cytosol, while ABA and GA3 exerted similar effect only after its growth for 1 h and 4 h, respectively. Kinetin, in contrast, resulted in acidification of the cytosol. Results indicate potential targets of the phytohormone action in pollen tubes.The important conclusion is that only in the case of auxin, all the observed hormone-induced responses of pollen tubes may be integrated into a common mechanism, in which Ca2+ ions can putatively serve as coordinative and integrating signal. Therefore, it can be concluded that auxin plays a key role in the maintenance of pollen tube polar growth that is in accordance with its similar behavior in other plant organs.

04. Transport

P-04-01

Arp2/3-dependent auxin transporter trafficking

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Plant morphogenesis and cell differentiation requires the coordination of the cytoskeleton and multiple signaling pathways. Arp2/3 complex is a conserved actin nucleator composed of seven subunits (ARP2, ARP3, ARPC1-ARPC5) whose mutation in plants leads to rather mild phenotypes, in comparison of those observed in animal and yeast.
Auxin is long known to be one of the main factors involved in the coordination of many cellular responses throughout plant development. Its activity relies in the generation of gradients across tissues. Auxin gradients are achieved by auxin transport by specific carriers asymmetrically distributed at the plasma membrane of cells within tissues. Auxin carrier targeting and trafficking relies, among others, on the actin cytoskeleton.
We have described previously that auxin distribution and basipetal transport is affected in Arabidopsis thaliana mutants lacking functional Arp2/3 complex. In this work we show that the Arp2/3 complex is involved in auxin transporters subcellular localization and recycling. We hypothesize that the mechanism of Arp2/3 function is based on Arp2/3 role in vesicle trafficking. These results contribute to the understanding of the defects in, for example, the gravitropic response of Arp2/3 mutants, which require fine tuning of auxin carrier localization in the plasma membrane.

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P-04-02

A novel cytokinin transporter controls legume-*Rhizobium* symbiosis

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Cytokinins (CKs) are ubiquitous plant hormones and signaling molecules. As phytohormones crucial for legume-*Rhizobium* associations, CKs play a role at early and later nodulation stages. In the root cortex, activation of cytokinin signaling pathway and as a consequence their biosynthesis/accumulation, triggers cell division and formation of root nodule. Interestingly, rhizodermal cytokinins are being suspected to act as a mobile signal joining outer and inner root tissue responses. However, dedicated transporters, mediating cytokinin translocation between rhizodermis and root cortex, as well as within cortical cell layers await discovery.
Here we present a root expressed full-size ABC (ATP-binding cassette) transporter from the G subfamily in the model legume plant *Medicago truncatula*. This transporter is expressed in the rhizodermis and root cortex and its mRNA accumulates upon symbiotic bacteria, isolated NF, as well as cytokinin treatments. Microsymbiont-dependent induction of its expression is restricted to the infection zone. The transporter is a plasma membrane protein and translocates bioactive cytokinins in an ATP-dependent manner. Disruption of this transporter results in the impairment of nodulation. In light of the presented data, a role of ABCG transporters in symbiotic interactions through CK translocation can be postulated.

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P-04-03

Silver ions increase plasma membrane permeability for various substances including auxins

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The action of silver ions at the plant plasma membrane is largely connected with the inhibition of ethylene signalling. Interestingly, it has been proposed a direct influence of AgNO3 on the auxin efflux while admitting that the molecular mechanism of this action remains unclear. Using tobacco BY-2 cells, we demonstrate that besides dramatic increase of efflux of synthetic auxins 2,4-dichlorophenoxyacetic acid and 1-naphtalene acetic acid, treatment with $AgNO_3$ resulted in enhanced efflux of the cytokinin *trans*-zeatin as well as the auxin structural analogues tryptophan and benzoic acid. The application of $AgNO_3$ was accompanied by gradual water loss and plasmolysis. The observed effects were dependent on the availability of extracellular calcium ions Ca^{2+}. Confocal microscopy of Ca^{2+}-sensitive fluorescence indicator Fluo-4FF, acetoxymethyl ester suggested that the extracellular Ca^{2+} availability is necessary to trigger a

response to silver ions and that an intracellular Ca^{2+} pool alone is not sufficient for this effect. Altogether, our data suggest that the effect of silver on the transmembrane transport in plants is not specific for auxin and that the effects of silver ions originate from the primal modification of the internal calcium levels, possibly by their interaction with Ca^{2+}-permeable channels at the plasma membrane.

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P-04-04

Unravelling the mechanism of PIN-mediated auxin transport

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Auxin and its directional distribution is important for the generation of developmentally instructive auxin gradients. The setting of these gradients within plant tissues depends on the activity and localization of auxin integral plasma membrane transporters. PIN-FORMED (PIN) auxin efflux carriers have been described to provide directional auxin transport throughout plant development. Within the PIN family, *Arabidopsis thaliana* PIN1 plays a crucial role and its mutation severely affects organ initiation, e.g. flower and leaf initiation. PIN1 topology predictions using bioinformatic analysis are insufficient to understand its auxin transport mechanism and there are only very few experimental data on the definition of domains mediating auxin transport. Therefore, from publicly available databases we have selected set of *pin1* mutants that probably produce aberrant protein products. We report on the characterization of a T-DNA insertion mutant *pin1* (N320510) with loss of function character. This line was shown to produce C-terminally trimmed product, had characteristic *pin1* mutant phenotypes and drastically reduced auxin transport in the inflorescence stems as well as reduced DR5::GFP maxima within the root tip. However, as shown by indirect anti-PIN1 immunofluorescence, both inflorescence stems and roots had mutated protein located at the plasma membrane in a BFA-sensitive manner. Our data indicates that C-terminus of PIN1 defines its auxin transport activity.

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P-04-05

Does differential plasma membrane distribution of *Nicotiana tabacum* PIN auxin efflux carriers defines their auxin transport function?

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The distribution of auxin carriers within the plasma membrane (PM) is still poorly understood. By the combination of fluorescence recovery after photobleaching (FRAP) and raster image correlation spectroscopy (RICS) we showed previously that speed of mobile fractions of AtPIN1 auxin efflux carrier and AtAUX1 auxin influx carriers depends on the composition of the PM. In this work, by the combination of confocal laser scanning microscopy (CLSM), spinning disc (SD) microscopy and variable angle epifluorescence microscopy (VAEM), we define PM distribution of three *Nicotiana tabacum* PIN auxin efflux carriers. On the transcriptional level, *NtPIN2* was found more in the exponential cells, while *NtPIN3* and *NtPIN11* in both exponential and stationary cells. GFP-tagged versions of *NtPINs* were expressed in tobacco BY-2 cells and RICS showed that speed of mobile fractions is comparable for all three PINs. However, by using FRAP, NtPIN11 is showed to have higher immobile fraction, both in the mature PMs and cell plates. Interestingly, auxin transport assays showed that NtPIN11 is the most effective in auxin efflux. The combination of SD, VAEM and super-resolution radial fluctuations (SRRF) algorithm image processing were used to show that PIN11 is often localized in the association with cortical cytoskeleton, while PIN2 shows evenly distributed domains. Our results suggest novel regulation of function for members of PIN family, based on their differential stabilization within PM.

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P-04-06

Biological characterization of new fluorescently labeled auxins

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The phytohormone auxin is a small molecule controlling most aspects of plant growth and development through the establishment of an auxin gradient in response to both inner and environmental stimuli. Coupling of an auxin molecule to a fluorescent probe provides a tool to visualize auxin *in vivo* distribution with an unprecedented spatiotemporal resolution. Here we present a biological characterization of two new fluorescent analogues of synthetic auxin 2,4–dichlorophenoxyacetic acid (2,4–D) including the evaluation of their time-course stability *in planta,* using ultra-sensitive liquid chromatography-mass spectrometry (LC-MS) method. Both compounds displayed negligible metabolization in roots of *Arabidopsis* and induced auxin-related responses in a short-time scale. After their uptake, these compounds display an uneven distribution observable as a fluorescence maximum in tissues such as the inner side of the apical hook or quiescent center (QC) cells of the root. These new fluorescent analogues represent new probes to instantly image auxin distribution during plant development.

P-04-07

ABP1 plays a role in post-transriptional control of PIN3 plasma membrane localization in roots of *Arabidopsis thaliana*

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Auxin Binding Protein 1 (ABP1) is studied for many years, but its involvement in the auxin signal transduction and auxin action is still not clear. Gene homologs for *ABP1* are found throughout green plant lineage from algae to angiosperms and the presence of ABP1 is characteristic for

meristems and rapidly growing tissues. While little is known about ABP1 physiological functions, its protein structure is well described. ABP1 is a small beta barrel with short alpha helix at the C-terminus, occurs as a dimer at the outer face of plasma membrane and was shown to bind auxin with high affinity. Using auxin transport assays, quantitative analysis of cellular morphology we previously showed that ABP1 regulates after its inducible overexpression auxin efflux from tobacco BY-2 cells. We pointed to the involvement of mechanism that involves the ABP1-controlled vesicle trafficking processes, including positive regulation of endocytosis of PIN auxin efflux carriers. Here we show by indirect immunofluorescence in CRISPR-generated knock out mutants of *Arabidopsis thaliana* (*abp1-c1*) and by *in vivo* studies in pPIN3::PIN3:GFP/*abp1-c1* that they both show light-dependent changes in the amount of PIN3 auxin efflux carrier in the plasma membrane. FRAP experiments suggested that the absence of ABP1 affected the speed of recovery of PIN3 within the plasma membrane. In contrast, qRT-PCR analyses showed no difference for *abp1-c1* and Col-0 pointing to the post-transcriptional character of observed effects. Altogether, our results point to the auxin-buffering role of ABP1 in the generation of developmentally important, PIN-dependent auxin gradients. Supported by CSF project GA16-10948S.

P-04-08

Study of the vacuolar and secreted cytokinin dehydrogenases of *Arabidopsis thaliana*, their influence on the cytokinin distribution in vacuoles and on the root system architecture

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Intracellular cytokinin homeostasis is maintained primarily by metabolic inactivation of the hormone, which is catalyzed by cytokinin oxidase/dehydrogenase (CKX). Arabidopsis CKX gene family is comprised of seven members, which differ in the subcellular localization of their protein products. CKX2, CKX4, CKX5, and CKX6 are processed by the plant secretory pathway, while CKX1 and CKX3 proteins are targeted to the vacuole and only CKX7 isoform is localized to the cytosol. Enhanced expression of *CKX* genes causes increased root growth phenotype. Transgenic *Arabidopsis* plants overexpressing *CKX1*, *CKX2* and *CKX3* and T-DNA knock-out lines *ckx2* and *ckx3* were used in this work as a tool for mapping the associated root architecture and characterizations of the total intracellular vs vacuolar pool of all cytokinin forms. Specifically, primary root length, number of lateral roots, and gravitropic set-point angles were measured in transgenic and control plants. The results showed that *CKX2* overexpressing plants produced the greatest number of lateral roots as well as the largest primary roots. In contrast, the cytokinin-deficient plants were characterized by a shift to a near vertical gravitropic set-point angle (GSA) of their lateral roots as compared to WT. The total quantities

of the intracellular and vacuolar content of cytokinins from *ckx2* and *ckx3* loss of function mutants, *CKX* overexpressing line, and Col-0 control were determined by UHPLC-MS/MS analysis. The results confirmed prevalence of the cytokinin storage forms (both *O*-glucosides and *N*-glucosides) in vacuoles. Interestingly, significant changes in the vacuolar pool of several cytokinin forms were observed in CKX3 but not in CKX1 lines. Our results confirm CKX3 as the main vacuolar isoform and contribute to better understanding of the mechanism of the cytokinin transport and storage in vacuoles.

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P-04-09

CRK5 kinase function in *Arabidopsis thaliana*

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The positive and negative gravitropic responses, in roots and shoots, are controlled by unequal distribution of auxin. The PIN2 protein plays crucial role in regulation of root gravitropic response; the PIN3 protein is a key regulator of shoot gravitropic and phototropic responses in stem endodermis, while PIN1 is involved in shoot and root development and the acropetally auxin transport from the shoot to the root. Basal-to-apical switching of the PIN proteins in cell membranes is stimulated by different kinases which can phosphorylate the PIN hydrophilic loops in different manner. Previously we have identified and characterized the CRK5 protein kinase. We showed that inactivation of CRK5 caused a root and shoot gravitropic defect, reduced root growth and enhanced lateral root formation. This phenotype is the result of altered auxin distribution caused by abnormal localization of PIN2 auxin efflux protein, which is phosphorylated by CRK5. Our preliminary studies suggest that relocation of PIN3 in stem cells might be influenced by CRK5 phosphorylation as well. In order to study this phenomenon we cloned the hydrophilic loop of PIN1, PIN2 and PIN3 proteins, and subsequently overexpressed and purified them from E. coli cells. Performing *in vitro* radioactive kinase assay we found that

the hydrophilic loop of all three PIN proteins was phosphorylated by CRK5 protein kinase. In cooperation with the Proteomics Research Group (BRC) we identified various possible phosphorylation sites. For mutant complementation and protein interaction studies we generated the gCRK5:GFP construct. After confirmation of functionality we used the plant lines for immunoprecipitation studies to find the interaction partners of the CRK5 protein kinase. In order to verify the possible interactions we used the BiFC method.

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P-04-10

PHOT1 phosphorylates PIN-LIKES to steer phototropic growth

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Plants are constantly exposed to different environmental conditions and light is among the most relevant environmental signals. Phototropism arises from an increase in auxin transport towards the shaded side, inducing cell elongation and, hence, hypocotyl bending towards light. Directional phototropic growth towards blue light is directed by the phototropins PHOT1 and PHOT2 in *Arabidopsis*. Although links between phototropism and auxin transport have been described, relatively little is known about the direct targets of the PHOT kinase. We used several approaches to identify kinases phosphorylating the ER-localized family of PIN-LIKES (PILS) putative auxin carriers (Barbez et al. 2012) and found that PHOT1 is able to directly phosphorylate a specific PILS proteins. We mapped the underlying phosphorylation sites and revealed that they modulate PILS function. In agreement, we show that the PHOT-dependent regulation of PILS impacts on phototropic growth towards blue light, but do not modify auxin-dependent growth towards gravity. Our data proposes a novel mechanism directly integrating blue light perception with intracellular auxin transport for differential growth responses.

P-04-11

Conserved tyrosine residues in the PIN central cytosolic loop are important for PIN polarity maintenance

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Polar transport of the plant hormone auxin is mediated by the PIN FORMED (PIN) auxin efflux carriers, which determine the direction of this transport through their asymmetric distribution at the plasma membrane. It is well established that PIN polar localization is altered by phosphorylation of these transporters at their central cytosolic loop by the AGC kinases PINOID, WAG1 and WAG2. However, how the phosphorylation status affects the PIN distribution is yet

unknown. Here we identified two conserved tyrosine residues located downstream of the AGC kinase target serines S1 and S2. Substitution of these tyrosines for alanines made PIN1 and PIN2 fluorescent protein fusions non-functional, in that they could not complement the corresponding loss-of-function mutants. Tyrosine to alanine substitutions affected binding of the CL to the ADAPTOR PROTEIN 1 and 2 mu subunits (AP1M2 and AP2M), and reduced the polar localization of PIN2. However, the mutations did not affect phosphorylation of the PIN CL by PINOID, nor did they change the effect of PINOID overexpression on PIN polarity. Our results suggest that the AGC kinase-induced polarity shift does not involve AP complex binding to these tyrosines, but that these conserved residues rather have a more general role in maintaining PIN polarity.

05. Interactions and cross-talk

P-05-01

Sugars trigger axillary bud outgrowth by impairing strigolactone perception in axillary buds

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Shoot branching and growth arising from the initiation and growth of axillary buds determines plant architecture. The shoot tip inhibits the growth of axillary buds hence shoot tip decapitation leads to rapid bud outgrowth. Rapid bud outgrowth observed within 3 hours after after decapitation in pea has been suggested to be sorely due to water influx into the buds. We hereby present evidence that d*e novo* protein biosynthesis is required for triggering axillary bud outgrowth confirming that proteins involved in catalytic activities and hormone signalling are involved in this process. Strigolactones (SLs) are the only known hormone class that inhibit axillary bud outgrowth. Inhibiting SL signalling with the synthetic SL perception inhibitor KOK1049 rapidly induces axillary bud outgrowth in pea plants by antagonizing D14/RMS3 proteins. However KOK1049 cannot induce bud growth on defoliated plants, while cytokinin (CK) applications and endogenous sucrose feeding rapidly induce axillary bud outgrowth on intact and defoliated plants. CKs and sucrose feeding are also capable of inducing axillary bud outgrowth when applied together with rac-GR24, a synthetic SL analogue. The expression of pea BRANCHED1 (BRC1), a TCP transcription factor restricted mostly to axillary buds whose activity inversely correlates with bud outgrowth, is repressed by CKs and sucrose even when applied together with rac-GR24. SL signalling defective mutant *rms3* pea is more sensitive to cytokinins and sugars relative to the wild type. These data suggests that an antagonistic interaction between sucrose and SLs regulates shoot branching, we therefore hypothesize that sucrose induces branching partially by repressing SL perception.

P-05-02

Ethylene-independent promotion of photomorphogenesis by cytokinin requires a functional cytokinin and light signaling pathway

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Seedlings germinated in the dark or in the light display fundamentally different growth patterns named skoto- and photomorphogenesis, respectively. Photomorphogenesis is induced by light and is characterized by the inhibition of hypocotyl elongation and opening of cotyledons. It is known that the plant hormone cytokinin, when applied in high concentrations, mimics the effect of light and inhibits hypocotyl elongation in dark-grown Arabidopsis seedlings. Previous work (Cary *et al.*, Plant Physiol. 1996) has suggested that this activity of cytokinin is largely mediated by ethylene. Here we show that treatment of etiolated seedlings by cytokinin in the presence of ethylene inhibitors (e.g. $AgNO_3$) or treatment of the ethylene-resistant mutant *ein2,* results in a significant inhibition of hypocotyl elongation, indicating that the cytokinin-induced de-etiolation is largely independent of ethylene. Triggering of the photomorphogenic response in darkness is mainly mediated through the cytokinin receptor ARABIDOPSIS HISTIDINE KINASE 3 (AHK3) and the ARABIDOPSIS RESPONSE REGULATOR 1 (ARR1) in combination with ARR12. Interestingly, mutation of the light signaling genes *COP1, DET1 and CIN4/COP10* renders dark-grown seedlings insensitive to cytokinin. These factors are also indispensable for the transcriptional response during cytokinin-induced de-etiolation, which indicates that a functional light signaling pathway is essential for this cytokinin response. In addition, the cytokinin effect on hypocotyl elongation is highly dependent on light conditions where higher light intensities cause a switch in the response to cytokinin from an inhibition to promotion of hypocotyl elongation. Together, these results suggest a close connection between the cytokinin two-component system and the light signaling network.

P-05-03

Dissecting the mechanism of cytokinin-ethylene crosstalk in the control of multistep phosphorelay signaling and its role in the root development

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Ethylene has been shown to be involved in controlling cytokinin-mediated root growth. Current research attempts to uncover several candidates that link between ethylene and cytokinin at the signaling level in the root. We have shown that ethylene contributes into the MSP pathway via histidine kinase activity of ETR1, controlling the activity of several response regulators (*ARRs*) (Zdarska et al, under revision).
To be able to study the effects of ethylene on the plant development and dissect ethylene-independent effects of the rate-limiting ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), we established a cultivation system operated by gas mixing system, allowing plant to be treated directly with ethylene gas inside sealed chambers and under defined light conditions. Our data show that alike of ACC treatment, 30 minutes of plant growth in ethylene-enriched atmosphere is able to induce *ARR3 gene* expression in the root tips. Using the system we examined several arr mutants in order to compare ACC- and ethylene-induced response of the root apical meristem (RAM) and to characterize the role of individual ARRs in the cytokinin-ethylene crosstalk in the root growth. Moreover, we identified another Arabidopsis histidine kinase that seems to be involved in the activation of MSP signaling in ethylene-, but not cytokinin-dependent manner. Furthermore, our data demonstrate differential role of canonical ethylene signaling components EIN2 and EIN3 in the ethylene-mediated MSP activation. Based on our findings, we propose that ethylene controls the MSP pathway at least at two different levels: via interaction of the ethylene-regulated sensor histidine kinases and at the level of ethylene-responsive transcription factors. The data showing both types of regulations will be presented.

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P-05-04

Brassinosteroids regulate expression of primary response genes to cytokinins in plants

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Brassinosteroids (BRs) are a group of steroid hormones of plants that regulated a wide range of development processes. The central to the hormonal control of plant growth and development

lie to the interaction of phytohormonal pathways. Indeed, brassinosteroids are participated in a complex signaling network via a modulation of the levels and sensitivity of other phytohormones or via the intersection of the primary signaling pathways. Some recent studies have identified the specific mechanisms of the coordinated action of BRs and several other phytohormones, including jasmonic acid, abscisic acid, gibberellic acid, auxin, and ethylene. The mechanisms of interplay between brassinosteroids and cytokinins are still obscure.
The goal of the study was to elucidate the influence of BRs on the expression of the genes participating in cytokinin signaling.
In the present study plants of *Arabidopsis thaliana* (L.) Heynh transformed with the PARR5::GUS construct and *Solanum tuberosum* plants we used to estimate the influences of several BRs (brassinolide, epibrassinolide and homobrassinolide) and 6-benzylaminopurine on the expression of the RR-A gene which belongs to the type A negative regulators of plant responses to cytokinin.
Our results demonstrate that the application of exogenous BRs induces the expression of the gene for the cytokinin primary response. The up-regulation of this gene by BRs occurred both in seedlings and mature leaves of potato and depended on the concentration of the hormone applied. However, the effect of BRs was substantially lower than that of 6-benzylaminopurine.
The results of our studies imply that brassinosteroids–cytokinins interactions are possibly mediated through various metabolic pathways and can be integrated in a complex signaling network.

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P-05-05

Chemical Spaces of Small Signal Molecules Inducing Biological Activity

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Our novel 'function-oriented pharmacophore' locates molecular features withing an oriented framework with n-dimensions instead to focus the analysis of an interaction ligand-receptor. The method connect and biochemical network signaling between two points of reference, the n-dimensional orientation of the small molecule structures (quantum chemical analysis) and their biological function. It could discriminate molecular properties responsible for functions and expression profiles at biochemical level. A theoretical analysis, backed by experimental observations, disclose different electronic features in small molecules that play an important role as condition for response networks of endogenous macromolecules.

P-05-06

Auxin Down-regulates *BAS1* Expression to Increase Endogenous Brassinosteroids in *Arabidopsis thaliana*

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BAS1 encodes CYP734A1, which inactivates the biologically active BRs via C-26 hydroxylation and is down-regulated by a BR-responsive transcription factor, BZR1. The expression of the *BAS1* is regulated by auxin response factors (ARFs) in *Arabidopsis thaliana*. Two successive E-box motifs on the *BAS1* promoter function as BZR1 binding sites and are essential for BR-regulated *BAS1*expression. The expression of *BAS1* is increased in the *arf7* and *arf7arf19* mutants. The endogenous level of a bioactive BR, castasterone, is greatly decreased in those mutants. ARF7 can bind to the E-box motifs of the *BAS1* promoter where BZR1 binds, suggesting that ARF7 and BZR1 mutually compete for the same *cis*-element of the *BAS1* promoter. Additionally, ARF7 directly interacts with BZR1, which inhibits their DNA binding activities and regulation of *BAS1* expression. In conclusion, auxin signaling via ARF7 directly modulates the expression of *BAS1* by competition with BZR1, thereby increasing the level of castasterone and promoting growth and development in *A. thaliana*.

P-05-07

Hormonal status and responsiveness to auxin and cytokinin of transgenic potato plants harboring *tms1* gene driven by tuber-specific promoter

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Previously, we generated potato transformants expressing *Agrobacterium*-derived auxin synthesis gene *tms1* driven by tuber-specific patatin gene (B33-)promoter. Here we studied endogenous hormonal status and response to exogenous phytohormones in *tms1*-transformants cultured in *vitro*. Adding IAA or kinetin to culture medium affected differently tuberization of *tms1*- and control plants, depending also on sucrose content in the medium. Exogenous phytohormones ceased to stimulate the tuber initiation in transformants at high (5-8%) sucrose concentration but not in control plants. Furthermore, auxin partly inhibited the tuber initiation, and cytokinin reduced the average tuber weight in most transformants at high sucrose. The

elevated IAA level in tubers of the transformants was accompanied with a decrease in content of cytokinin-bases and their ribosides. No concerted changes in contents of abscisic, jasmonic, salicylic acids and gibberellins in tubers were detected. This indicated that the enhanced productivity of *tms1*-transformants was due to auxin and not mediated by other phytohormones. In addition, exogenous cytokinin was shown to upregulate the expression of genes encoding orthologs of auxin receptors. Overall, the results showed that local increase in IAA level in transformants affects both the balance of endogenous cytokinins and the dynamics of tuberization in response to exogenous auxin or cytokinin, the latter reaction depending also on the carbohydrate supply. We introduce an updated model for the hormonal network controlling tuberization.

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P-05-08

Importance of sensitivity to ethylene for the control of auxin and cytokinins content and growth of Arabidopsis plants

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Ethylene is known to control various processes by regulating biosynthesis and distribution of other plant hormones. To check this assumption we compared IAA and cytokinins (CKs) content in WT (COL) *Arabidopsis* plants and their ethylene insensitive *etr1-1* mutant. The mutant grown in sand supplied with the mineral nutrients was distinguished by elevated concentration of IAA in both roots and shoots and of CKs in the roots as compared to the COL. Higher concentration of IAA in the roots of *etr1-1* was supported by the intensification of GUS-staining of the roots of transgenic plants transformed with auxin sensitive construction and treated with the inhibitor of ethylene perception (MCP). Elevated level of hormones in the mutant were far more noticeable in plants supplied with sufficient amount of nutrients, when they produced more ethylene as compared to the nutrient-starved plants. The roots of *etr1-1* were longer than those of COL despite elevated concentration of IAA and CKs in them. Elongation of *etr1-1* roots was insensitive to the exogenous IAA shown to inhibit elongation in COL. This effect suggests direct impact of ethylene on the process resulting in longer roots of the mutant. The effect of ethylene insensitivity on root mass accumulation was opposite to that of root elongation, and COL roots were heavier than those of *etr1-1*. The effect may be attributed to elevated CK accumulation in the roots of the mutant, since CKs are known to inhibit root growth. Higher concentration of CKs in *etr1-1* as compared to COL was likely to be due to reduced activity of their conjugation suggested by decreased level of CK glucosides. Benzyladenine decreased root biomass of both genotypes confirming preservation of sensitivity to CKs in terms of biomass accumulation in the

mutant. Elevated content of IAA and CKs in *etr1-1* plants suggests importance of ethylene for preventing their excessive accumulation and enabling adequate plant growth.

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P-05-09

The effect of GR24 on physiological responses of *Arabidopsis thaliana* in dependence on phosphate nutrition

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Inorganic phosphate (Pi) is one of the most important nutrients for plants and its lack or inaccesibility is considered a major abiotic stressor. Unfavourable environmental conditions may lead to oxidative stress and subsequent damage. Strigolactones (SLs) represent a group of phytohormones influencing many growth and developmental processes. Apart from shoot branching inhibition, their role in regulation of plant responses during Pi deficiency has been documented.

The effect of synthetic SL analog GR24 on selected growth parameters, Pi uptake and lipid peroxidation (as a marker of oxidative stress) was investigated in *Arabidopsis thaliana* during Pi starvation and in full Pi nutrition (100 µM). Concentration range 0 – 5 µM GR24 was used in 7-day experiment.

Decrease of shoot/root ratio after application of GR24 was observed. GR24 enhanced Pi uptake, at concentration 1 µM significantly, at 5 µM GR24 very strongly. Leaves supplemented with Pi exhibited a clear positive correlation between GR24 concentration and lipid peroxidation level. This might be attributed to SL signalling for remobilization of Pi from phospholipids in cell membranes, irrespective of Pi nutrition. The other posibility might be a slightly toxic effect of higher GR24 concentrations. Nonetheless, only 1 µM GR24 led to a significant difference in lipid peroxidation in dependence of Pi nutrition.

SLs exhibit a cross-talk with other phytohormones such as auxins and cytokinins. They influence auxin transport affecting PIN proteins. GR24 was found to down-regulate the content of the most physiologically active cytokinin *trans*-zeatin in whole plant increasing *cis*–zeatin in roots. GR24 was also proved to have a concentration dependent effect on shoot/root ratio, lipid peroxidation and Pi uptake. Namely, 1 µM GR24 seemed to exhibit the most contrasting effects on physiological responses in dependence on Pi nutrition.

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P-05-10

Metabolism and transport of cytokinins in stressed plants and importance of abscisic acid for their control.

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Cytokinins are necessary for normal shoot growth, and the decline in their content contributes to the inhibition of shoot growth in stressed plants bringing about a release of resources for adaptive responses. We addressed mechanisms implicated in the control of cytokinin content and their relation to accumulation of abscisic acid (ABA) in stressed plants. Dilution of the nutrient solution of wheat plants decreased cytokinins due the elevated activity of cytokinin oxidases. This process was associated with accumulation of ABA suggested by disappearance of the cytokinin responses in plants pre-treated with the inhibitor of ABA synthesis. The effect was not universal and the decline in shoot cytokinins was not coupled with activation of cytokinins oxidase in P-starved barley plants. In this case, the drop in shoot cytokinins was accompanied by weaker immunostaining for cytokinins and greater staining for ABA of root tips. The absence of cytokinin decline in either shoots or root tips of ABA deficient barley plants suggested involvement of ABA in cytokinin responses to P-starvation. Next novel mechanism implicated in the control of shoot cytokinins in stressed plants was detected in wheat exposed to increased ostmotic potential of the nutrient medium. In this case the decline in shoot cytokinins was brought about by inhibition of their xylem transport from the roots. Retention of cytokinins in the roots was due to their active uptake by the root cells suggested by a weakened immunostaining for cytokinins of root cells treated with protonophore that destroys proton gradient (the main source of energy for the cytokinin uptake). Protonophore-treatment increased delivery of cytokinins to the shoots thereby preventing the decline in their content in the stressed plants. The results suggest importance of active cytokinin uptake by root cells as one of mechanisms controlling cytokinin delivery to the shoots of stressed plants.

P-05-11

Linking the *Arabidopsis* response regulator proteins to the transcriptional network

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Cytokinin is one of the important phytohormones regulating various aspects in plant life. Cytokinin is sensed by histidine kinase receptors, which are transmitting the signal by a histidine/aspartate phosphorelay system to the phosphotransmitter proteins (HPts) and finally B-type response regulators (RRs). B-type RRs are transcription factors regulating the expression of cytokinin response genes including A-type *ARR*s, negative regulators of the signaling pathway. Together with other effector proteins, A-type and B-type ARR determine the cytokinin signaling output. The diversity of cytokinin functions makes it necessary to interconnect the pathway with other signaling pathways and to fine-tune the activity of the ARR proteins. It has been shown that B-type response regulator proteins (ARR) interact with other transcription factors which in turn modulate their activity, e.g. the DELLA proteins, negative regulators of the gibberellin signaling pathway. It is likely that more transcription factors interact with B-type ARRs to modulate their activity. We have therefore started to analyze putative interaction partners of B-type ARRs by screening approximately 1500 transcription factors, cDNA TF library of Mitsuda *et al.* (Plant Cell Physiol., 2010), with the yeast two hybrid system. First results show that B-type ARR proteins interact with a wide range of different transcription factors representing various signaling pathways. Our results suggest that the cytokinin signaling output is fine-tuned by the interaction with different transcription factors. Progress on the emerging interaction network will be reported and discussed.

P-05-12

Changes in the light spectral quality affects cytokinin homeostasis, regulating the senescence rate in wheat leaves exposed to shading stress

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Suppression of blue light (BL) markedly accelerates the senescence rate in wheat leaves under shading. Previous studies have shown that BL contributes to maintain a higher level of certain endogenous cytokinins (CKs), although the mechanism involved was not elucidated. In order to better understand the role of BL and CK-metabolism in the control of senescence, detached leaves of wheat were exposed to shading stress under selective filters with low (green filter, G) or high transmittance (blue filter, B) in the blue region of the spectrum. A neutral filter (NF) was used as a control. Changes in chlorophyll (chl) content, cytokinin oxidase/dehydrogenase (CKX) activity and the expression profile of senescence markers and genes related to CK- reception, -signal transduction and –turnover were analyzed at different time points. CK-degradation, as evidenced by changes in CKX activity and the expression profile of *TaCKX1*, was markedly up-regulated as senescence progressed, particularly in leaves exposed to filter G. Treatment with INCYDE (an inhibitor of CKX) consistently retarded chl degradation in the absence of BL. The level of *TaZOG2* transcripts increased at a higher rate in the absence of BL, which would favor

CK inactivation through O-glycosylation. On the other hand, the expression of *TaGLU1c*, a gene involved in CK re-activation, was markedly up-regulated at 54 h and it maintained at higher levels than the initials in treatment B until the end of the experimental period. Interestingly, leaves from treatment B showed a significant increment in the expression of *TaHY5*, which encodes for a transcription factor that acts downstream of blue light receptors and interacts with CK metabolism in the regulation of some developmental processes. According to our results, both the maintenance of the homeostasis of active CKs as well as the stimulation of key transcription factors might be involved in the senescence-retardant effect exerted by BL.

P-05-13

Identification and quantitative measurement of proteins of biosynthesis and signaling plant hormones concerning apical dominance using MRM assays by mass spectrometry

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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. To date, we demonstrated that auxin negatively regulates local CK biosynthesis in the nodal stem by controlling the expression level of *PsIPT*. Furthermore, before and several hours after decapitation, *PsIPT*, *PsCKX*, *PsAD1*, *PsPIN1*, *PsYUC* and *PsBRC1* expressions were markedly increased and deceased in the nodal stem and axillary bud using analyses on qRT-PCR. However, we cannot detect each protein change using Western blotting by each antibody because each protein content level was very low. Here, We have developed a highly sensitive analysis for protein identification and quantitative measurement of proteins based on multiple reaction monitoring (MRM) assays by mass spectrometry. Our method, depended on the report by Matsumoto et.al (Nature Methods, 14: 251, 2017), comprised MRM assays of mass tag (mTRAQ)-labeled peptides to measure the abundance of target proteins using LC/MS/MS analysis coupled triple quadrupole mass spectrometer (QTRAP 5500 Sciex). We used the target sample tryptic peptides labeled by mTRAQ Δ0 and recombinant ones as the internal standard labeled by mTRAQ Δ4. Theses mTRAQ labeled peptides were combined and then analyzed MRM assay by mass spectrometry. As the result of this method, several proteins were detected and quantitatively measured.

P-05-14

Cytokinin production in soybean roots differs between soybean cyst nematode susceptible and resistant cultivars

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Sedentary endoparasite soybean cyst nematode (SCN) (*Heterodera glycine* L.), causes a great loss in soybean (*Glycine max* L.) production in North America. A group of phytohormones cytokinins (CKs), plays a key role in the development of the cyst nematode during soybean root infestation. It has been previously reported that both host and pathogen contribute to CKs production to mediate the host-pathogen interaction that results in the formation of a hypermetabolic feeding site (syncytium). In this study**,** we investigated CK profile differences in SCN susceptible and resistant soybean cultivars over time. It was hypothesized that susceptible soybean cultivars produce more active CK forms than the resistant cultivars during early plant developmental stages and maintain the high production till maturation. Thus, susceptible cultivars facilitate the formation of syncytium. To identify the differences in CK profiles, the lateral roots from two pairs of susceptible and resistant, non-infested soybean cultivars at three developmental stages (VE = 5DAS, V1=14DAS, R1= 40DAS) were analysed. Regardless of SCN susceptibility, total CK level in roots of all tested cultivars decreased significantly from vegetative emergence (VE) to reproductive stage (R1) (191.95-224.05 pmol/g FW at VE stage and 9.67-25.97 pmol/g FW at R1 stage). Additionally, no notable differences in the level of active CK forms (free bases; FB) were found between susceptible and resistant cultivars at first two tested vegetative stages (VE and V1). However, during the reproductive stage (R1) susceptible cultivars still maintained high levels of FBs (>20 pmol/g FW), while no FBs were detected in the roots of two resistant cultivars.

P-05-15

Methylation of mRNA is required for auxin dependent processes

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Increasing number of evidences demonstrates that regulation of various steps of mRNA maturation influences auxin response. *N6*-adenosine methylation of mRNA (m^6A) is important posttranslational modification, which receives increased attention in past years, but its purpose in plants is practically unknown. We have recently identified components required for m^6A

in *Arabidopsis* and characterized phenotypes associated with this modification. In our further work, we found that depletion of m^6A is associated also with a range of auxin related defects. Moreover, mutants with low m^6A levels show strong resistance to exogenously applied auxins. We extensively compared the phenotypic defects of these mutants with collection of other known lines carrying both defects in mRNA maturation and auxin dependent processes. The phenotypic similarities suggest that auxin related defects in mutants with depleted m^6A levels might be linked with nuclear poly(A) polymerases. We thus hypothesize that m^6A in plant might be linked with poly(A) choice in mRNA.

P-05-16

Heat-induced male sterility is reversed by cytokinin, mediated by sucrose and expression of sugar transporter AtSweet 7

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High temperatures during flowering are known to reduce reproductive success in plants due to male sterility. This involves reductions in pollen production, viability, release, or growth of pollen tubes. Molecular and hormonal mechanisms behind heat sterility are not well understood. Existing evidence indicates cytokinins are essential to pollen production at normal temperatures in several plant models. Specific to high temperatures, sugars are implicated in reproductive success in tomato. We show here that exogenous application of cytokinins, as well as of sucrose, substantially improved fertilization and fruit set ($P<0.05$) under high temperatures in *Arabidopsis thaliana*. In multiple trials of bean and maize under high flowering temperatures in the field, reproductive success and yield were also increased ($P<0.05$) by cytokinin. A mechanism behind this is proposed to involve sugar movement to and accumulation in flowers. Consistent with this, cytokinin application rescued heat-induced repression of the sugar transporter AtSweet 7 in *Arabidopsis*, and an AtSweet 7 null line showed reduced recovery of heat fertility by cytokinin treatment compared to wild type.

P-05-17

Molecular mechanisms of cytokinin-regulated endomembrane trafficking to coordinate plant organogenesis

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Plants build an extensive root system as a result of recurrent initiation and development of lateral organs that enables effective exploitation of soil nutrients. Lateral root organogenesis is under control of complex regulatory networks including plant hormones. Auxin and cytokinin are key hormonal regulators of this developmental process. Whereas auxin acts as a positive regulator of lateral root initiation and development, cytokinin suppresses both phases of lateral root organogenesis. This implies that their activities must be tightly coordinated.
In our previous work we have shown that part of the cytokinin-regulated root system establishment involves modulation of the auxin efflux carrier PIN1 trafficking and its redirection for lytic degradation into vacuoles (Marhavy 2011, 2014). To assess molecular principles of this alternative mode of cytokinin action that uses endocytic trafficking as a means to regulate plant organogenesis we performed a phosphoproteome analysis of *Arabidopsis thaliana* roots upon treatment with cytokinin. Using label-free quantitative (phospho)proteomics we identified proteins which in response to cytokinin exhibited a significantly changed abundance or phosphorylation pattern. Among candidates, proteins implicated in the endomembrane trafficking which exhibit either a significant change in their phosphorylation pattern and/or abundance are of primary interest and their potential role in cytokinin-triggered PIN1 lytic degradation will be discussed.
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P-05-18

Targeted plant hormone analysis in sorted cell populations – method development

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Plant hormones are signalling molecules and acting as chemical messengers they are involved in all plant developmental processes. The occurrence and the levels of these compounds strongly depend on plant organ, age, developmental stage and environmental conditions. Plant hormones are an extremely large family of diverse compounds which could be divided into several structurally different groups such as purine and indole derivatives, plant steroids, lipid-based substances, and terpenoid carboxylic acids. A common characteristic for all these

substances is their low abundance in plant tissues (10^{-10} – 10^{-15} mol/g fresh weight). Thus, their direct quantification in very complex plant extracts poses a challenging analytical task.
In this study, we focus on development of a new fast protocol including both extraction and analysis of multiple compounds from several plant hormone groups (cytokinins, auxins, salicylates, abscisates and jasmonates) in sorted cell populations. Utilization of fluorescent-activated cell sorting, with combination to liquid-liquid extraction, followed by in-tip solid phase purification and sensitive ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) should enable us to carry out this analysis in very small samples, counting 50 000 to 200 000 sorted cells. Using this newly developed approach we expect to gain a broader insight to plant hormone homeostasis with emphasis to precise localization of these compounds in difficult-to-isolate tissues (e.g. founder cells, QC).

P-05-19

Functional and structural insights into the mechanism of ETR1-mediated cytokinin-ethylene crosstalk

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Our recent data suggest important role of histidine kinase (HK) activity of ETHYLENE RESPONSE1 (ETR1) in the ethylene-dependent control of multistep phosphorelay (MSP) signaling (presented on the event by Zdarska et al.). However, the mechanism of ETR1-regulated phosphorelay is unclear.
We found out that receiver domain (RD) of ETR1 ($ETR1_{RD}$) does not bind divalent ions and lacks slow dynamics in its γ-loop, both demonstrated to be necessary for the ability of known RDs to activate MSP response. Structural analysis revealed atypical orientation of $ETR1_{RD}$ γ-loop that might result in the inability of $ETR1_{RD}$ to accept phosphate. In line with that, results of our in vitro kinase assay revealed that $ETR1_{RD}$ is not phosphorylatable by its HK domain ($ETR1_{HK}$). To attempt phosphorylation-mediated activation of $ETR1_{RD}$, a set of $ETR1_{RD}$ mutants with the γ-loop designed according to the sequence of functional homologous RDs were prepared. However, all the mutants were still unable to receive phosphate. X-ray crystallography showed no changes in the 3D structures of the $ETR1_{RD}$mutants, suggesting that the abnormal positioning and observed changes in the conformational dynamics of $ETR1_{RD}$ are encoded also in the other portions of the domain. On the other hand, mutations in only two amino acids of the γ-loop of $CKI1_{RD}$ mimicking the sequence of $ETR1_{RD}$ disturbed its conformational behavior and impaired the ability of $CKI1_{RD}$ to accept phosphate. Employing the in vitro kinase assay, we

identified receiver domain of one of the Arabidopsis HKs (AHK_{RD}) to be able to accept the phosphate group from $ETR1_{HK}$ and transfer it to AHPs, the downstream members of the MSP pathway in order of seconds, suggesting the AHK_{RD} as a cognate partner of $ETR1_{HK}$. Altogether, our results suggest phosphorylation-independent role of $ETR1_{RD}$ and crosstalk of ETR1with cytokinin signaling pathway via ETR1-mediated phosphorylation of another AHK.

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P-05-20

Study of isoprenoid-derived plant signalling molecules during the ontogenesis of spinach (*Spinacia oleracea* L.)

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Spinach (*Spinacia oleracea* L.) is a very significant leafy vegetable for human health mainly for its high content of carotenoids, vitamin C and B9. Among carotenoids, spinach contains high levels of lutein and zeaxanthin that are selectively deposited in the retina of the eye and help to prevent eye diseases such as cataracts. In addition to carotenoids, for which spinach is mainly grown, its plants also contain not less significant quantities of minerals such as calcium, iron and magnesium. Spinach also belongs to the limited number of plant species in human diet having capacity to produce phytoecdysteroids (PEs). These substances are considered as plant secondary metabolites protecting plants against non-adapted insects and nematodes. Regarding mammals and humans, PEs attract great attention for their potent pharmacological properties including performance-enhancing and wound healing effects. The aim of this study was to investigate the dynamics of PEs content in selected variety of spinach during early developmental stages of a plant. It is known that these processes are mainly driven by some plant signalling molecules like plant hormones. Plants' intricate signalling networks are further complicated by links and interactions with synthesis and metabolic pathways of secondary metabolites. To study these interactions, we performed LC-MS based targeted profiling PEs as the representatives of secondary metabolites together with profiling other isoprenoid-derived plant signalling molecules belonging to plant hormones such as cytokinins (CKs) and gibberellins (GAs). Presented work represents a pilot survey focused on crosstalk between the endogenous isoprenoid-derived plant chemical messengers whose the key C_5 isoprenoid building units IPP and DMAPP are produced in plant cell by two known biosynthetic pathways: the plastidial methylerythritol phosphate pathway (GAs, *trans*-zeatin and isopentenyladenine CKs), and cytosolic mevalonate pathway (PEs, *cis*-zeatin CKs).

P-05-21

The crosstalk between phytohormones and polyamines regulate plant stress tolerance

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Polyamines (PAs) are small ubiquitous molecules involved in many biological processes in plants including the response to a variety of unfavorable environmental conditions. The exogenous application of PAs is used as an effective approach for enhancing tolerance and productivity of crops under stress conditions. Plant stress responses have been also correlated with changes in hormone levels and signaling, where the pathway of PAs is included. However, it is not clear whether the crosstalk between PAs and hormones modulates plant stress tolerance. Therefore, it is important to quantify the changes in the levels of hormones and/ or PAs to stablish the interconnection between them and mainly to identify the candidate metabolites involved in plant stress tolerance. To improve our understanding of plant stress response, we have recently developed a simple and rapid method for qualitative and quantitative analysis of 12 PAs including the separation of isomers such as spermine and thermospermine using ultra-performance liquid chromatography (UPLC) coupled to a tandem quadrupole mass spectrometer (MS/MS) equipped with an electrospray interface (ESI). The samples were derivatized by benzoyl chloride and separated with C18 columns by a gradient elution with methanol – water. The proposed procedure allows a rapid, sensitive qualitative and / or quantitative analysis of PAs in different plant tissues with high performance, robustness and accuracy. In future, the quantification is going to be improved by use of polyamines' internal standards, which are currently being synthetized.

P-05-22

Auxin and cytokinin metabolic profiling of tomato flower and early fruit development

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The interaction(s) between auxins (AUXs) and cytokinins (CKs) are known to control plant reproductive organ development; especially gynoecium patterning. To obtain a more complex picture of AUX/CK status, in cross-regulation(s) of other hormones a maiden UPLC-ESI-MS/MS-driven phytohormone profiling in coupled with transcriptome analysis of hormone-related genes have been performed during tomato generative organs development. Hormonal detection was carried out in different floral organs (sepals, petals, stamen, pollen, carpels) at multiple stages of development (3, 5, 7 and 10 mm of bud length and at anthesis); and also in early fruit tissues (ovary, skin+pericarp, jelly, placenta, seeds) tissues at different developmental stages (5, 7, 10 and 15 days after anthesis[DAA]) tissues. metabolites Isopentenyl adenine and its derivatives were the most abundant among 25 detected CK. The highest CKs-abundance was observed in stamen of 5-7 mm flower bud, indicating the possible involvement of these hormones into microsporal development. At the same time, the content of CK-nucleotides and trans-zeatin riboside (TZR) was found to be induced during carpel development. The same experimental setup reflects a decreasing gradient of AUXs metabolites across all the flower tissues. The most noticeable AUXs recession was observed in stamen, thus pointing out on the antagonism with CKs pattern, that can indicate on the tight cross-talk between the hormones during flower male organ development. After the anthesis the CKs profile changed towards TZR domination in the majority of investigated tissues, but the most pronouncedly – in the seeds. The content of AUXs in young fruits decreased to the 15 DAA in skin/flesh with synchronous accumulation in seeds. Such crucial changes in AUXs CKs metabolism during tomato reproductive organs formation were linked to the spatiotemporal changes in the expression of genes, involved in biosynthesis/inactivation, transport and signaling of the hormones.

P-05-23

A reverse genetics approach to discover novel regulators of cytokinin biosynthesis and signaling in the shoot apical meristem of *Arabidopsis thaliana*

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Cytokinins were first studied for their ability to promote cell division in conjunction with Auxin. There is extensive evidence that cytokinin signalling plays key role in shoot and root patterning, senescence and sensing environmental signals. However, these studies do not elucidate the mechanism behind the maintenance of homeostasis in cytokinin biosynthesis and signalling. Genetic studies have shown that cytokinins are positive regulators of meristematic activity in

the shoot and any change in the level of cytokinin has a direct effect on the size of the shoot apical meristem (SAM). Despite having comprehensive knowledge of cytokinin biosynthesis and signalling, very little is known how the genes encoding receptors, biosynthesis enzymes, and degradation enzymes are regulated at single cell and tissue level. In this study, we identified the genes involved in cytokinin biosynthesis and signalling from cell type specific microarray study and cloned their 3-kb upstream regulatory sequence. The promoters of cytokinin biosynthesis and signalling genes were used as bait against the shoot-enriched transcription factors (TFs) prey proteins to identify the potential regulators. We have screened 35 promoter baits against 350 TF preys and were able to find 172 interactions. The TFs that are interacting with these promoters are known to be involved in controlling developmental processes, abiotic stress and hormone crosstalk. There is a high representation of TFs that are induced by abiotic stress and are binding to the biosynthesis and signalling genes at multiple levels. Although it is well understood that abiotic stress leads to a reduction in cytokinin levels, the factors responsible for this shift have not yet been characterised. By validating these interactions *in-vivo* using genetic and molecular approaches, we aim to understand how cytokinin homeostasis is modulated by environmental cues and the consequence of this on the maintenance of the shoot apical meristem.

P-05-24

ETR1 and ARR3 interconnects ethylene and cytokinin into a single multistep phosphorelay pathway to control root growth

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Plant hormones cytokinins and ethylene modulate key developmental responses in *Arabidopsis*. Both hormones are recognized by receptors from the sensor histidine kinase (HK) family but act through distinct signaling pathways. The importance of cytokinin-ethylene signaling crosstalk has been proposed, however, the experimental evidence is still incomplete.
Our data display that not only cytokinins but also ethylene is able to control root apical meristem size (RAM) by activating the multistep phosphorelay (MSP) pathway. Ethylene-responsive ETHYLENE RESPONSE 1 (ETR1) contributes to the cytokinin-induced MSP output in the root and ETR1 signaling is essential for the early RAM response to both cytokinin and

ethylene. We also demonstrate that the HK activity of ETR1 play a crucial role in the ethylene-mediated RAM shortening.
We show that not only cytokinin but also ethylene controls MSP signaling in the root tip. We reveal that both hormones can act in similar but also in different cell types. Namely, ETR1-mediated ethylene signaling controls MSP primarily in the root transition zone. Via analysis of individual *ARABIDOPSIS RESPONSE REGULATORS TYPE A* (*ARRs-A)* we recognize several early MSP targets specific and common to both hormones. Further, we show that ethylene-induced *ARR3* controls RAM size. We also find that ETR1-dependent MSP signaling in the root tip spatially differs from canonical CTR1/EIN2/EIN3 ethylene signaling, indicating the developmental effects of both pathways can be spatially relevant.
In support of the role of ETR1 in MSP signaling we show that receiver domain of ETR1 interacts with ARABIDOPSIS HISTIDINE-CONTAINING PHOSPHOTRANSFER PROTEINS (AHPs), the MSP signaling intermediates acting downstream of cytokinin receptors. Based on these data we propose that MSP incorporate signal inputs from both cytokinin and ethylene to control cell differentiation in particular root zones.

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06. Interaction with the Environment

P-06-01

U. maydis proteins induce auxin signaling by targeting a key regulator of auxin signaling

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The biotrophic fungal pathogen *Ustilago maydis* infects maize. Successful infection depends on a battery of effector molecules delivered by the pathogen into the host during colonization. Effectors facilitate the entry and spread of the fungus, suppress immunity and re-shape the host's metabolism for the pathogen's benefit. It was previously shown that *U. maydis* synthesizes auxin (I3AA – Indole-3-acetic) during infection, substantially elevating I3AA levels in the colonized plant tissues. However, our preliminary results indicate that an auxin biosynthesis-deficient *U. maydis* strain still activates auxin signaling in infected plant tissue, suggesting an additional mechanism of activating auxin signaling.
In a candidate screen we identified secreted candidate effector proteins of *U. maydis* which can activate the auxin signaling of *Nicotiana benthamiana*. Using Co-IP coupled mass spectrometry

and yeast two hybrid screening, we identified a novel, functional family of *U. maydis* effector candidates, so called TIPs, which activate auxin signaling in diverse plant species by interacting with a known component of the auxin signaling pathway.
Our current aim is to elucidate the molecular mechanism by which TIPs activate host auxin signaling and how it affects the establishment of the *U. maydis* - maize interaction.

P-06-02
The role of cytokinin in the response to altered photoperiod stress

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Plants, as sessile organisms, have to cope in a fast and efficient manner with environmental cues to ensure their survival. Recently, a novel type of abiotic stress caused by a changed light-dark cycle - coined altered photoperiod stress - has been described. Characteristic for the response to altered photoperiod stress is the induction of stress and cell death marker genes, the accumulation of jasmonic acid, a strongly reduced photosynthetic efficiency and programmed cell death in leaves. The phytohormone cytokinin has been demonstrated to act as a protectant against the consequences of altered photoperiod stress. Here, we tested the stress response of a variety of cytokinin metabolism and signaling mutants that led to the identification of a cytokinin stress response pathway. In addition, the altered photoperiod stress responsiveness of leaves of different age and developmental stage was evaluated. Results indicate that leaves acquire the competence to perceive and respond to altered photoperiod stress over time independent of the age pathway.

P-06-03
Characterization of the impact of stress targeting and acclimation on heat shock response

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Plant responses to heat stress (HS) are highly influenced by stress targeting (stress application to shoots, roots or to the whole plant) as well as by acclimation pre-treatment. In this study on rice (*Oryza sativa*), HS targeting to roots had positive effect on maximum quantum yield of photosystem II in leaves (accompanied with lower non-photochemical quenching) associated with transient elevation of cytokinin signal transduction in non-stressed organ(s). HS targeting to shoots resulted in decrease of maximum quantum yield and elevation of non-photochemical quenching. HS exposure of whole plants had the strongest negative effect on photosynthesis. HS had relatively minor effect on membrane lipid peroxidation in roots, the most profound differences were found in crowns, in which HS application to whole plant led to substantial damage. Acclimation diminished the negative effect of HS both on quantum yield and on hormone stress responses. Only mild elevation of *cis*-zeatin and its riboside was determined in pre-acclimated leaves in contrast to direct HS application. The strongest HS response in roots was associated with drop of jasmonic acid. Acclimation also diminished stress induced elevation of abscisic acid and indole-3-acetic acid.
HS response is highly organ specific, depending significantly on stress targeting as well as on acclimation.

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P-06-04

The interplay of light, cytokinins and cytokinin receptors during senescence of detached leaves

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Cytokinins are well-known for their ability to retard or postpone senescence – the final stage of leaf development, which is essentially connected with an increase in lipid peroxidation and a decrease in chlorophyll content and photosynthetic activity. On the other hand, numerous

evidences indicate diverse results of cytokinin action. The inconsistencies are often associated with various experimental conditions but deeper understanding of cytokinin interaction with the environment is still missing. We tested the effect of cytokinins on leaf segments of barley, tobacco, lettuce or *Arabidopsis* under different light conditions and found that light radically affects the result of cytokinin action. In order to understand the interplay between cytokinin and light more deeply, we applied a cytokinin (0, 10^{-7}, 10^{-6}or 10^{-5} M 6-benzylaminopurine, BAP) on detached leaves of *Arabidopsis* double-mutants which have functional only one of the three known cytokinin receptors (AHK2, AHK3 or AHK4). Leaves of these mutants were kept for six days under various light conditions and changes in levels of endogenous cytokinins, photosynthetic performance and lipid peroxidation were analysed. We found that the threshold concentration of BAP at which the retarding effect on senescence switches to the accelerating one is different for different parameters and that this threshold shifts with the change of light intensity. Our study further demonstrates that while AHK3 was the main receptor mediating the effect of cytokinins on chlorophyll content and photosynthetic function, AHK4 primarily mediated the cytokinin effect on lipid peroxidation. AHK2 mediated both of these effects, but only partially.

P-06-05

Characterisation of *Medicago truncatula* root specific ABC transporter modulating lateral root density and nodule number

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Cytokinins are involved in a wide variety of plant growth and development processes as well as responses to environmental cues. They play an important role in the root architecture. In legumes, cytokinins control lateral roots outgrowth and participate in the onset of root cortical cell division, which lead to the formation of nodules. Initiation of lateral root and nodule formation is influenced by environmental stresses, namely nitrogen starvation and drought. Both conditions affect nodules and lateral roots in opposite manner. While nitrogen shortage increases number of nodules and decreases lateral roots density, drought suppresses nodulation and supports lateral roots emergence. Here we present an ABC transporter found in *Medicago truncatula* root vascular bundles, as well as apical meristem of root and nodules. Expression of this transporter is upregulated by (i) exogenously applied cytokinins, (ii) nitrogen limitation and (iii) *Sinorhizobium meliloti*. Its expression is also down regulated upon abscisic acid treatment and drought stress. Upon nitrogen limitation plants without this transporter are characterized by lower lateral root density and nodule number. We propose that this transporter is implicated in negative regulation of nodulation and lateral root formation.

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P-06-06

Cytokinin-producing, drought-tolerant *Methylobacterium* improves growth and yield characteristics of lentil (*Lens culinaris*) under water stress conditions

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Phytohormones regulate multiple processes of plant metabolism, including in response to biotic and abiotic stresses. Cytokinins (CK) are responsible for cell division, nutrient allocation and enhancement of photosynthesis. Increasing the levels of CK through the exogenous hormone treatment or genetic modifications can reduce negative effects of limited water availability. We investigated the effect of an increased pool of naturally produced CK in lentil (*Lens culinaris*) exposed to drought during the growing season. The increased CK was achieved by inoculating plants with growth promoting *Methylobacterium*, a beneficial plant endophyte capable of synthesizing high levels of CK, including the most active free base form, Zeatin. *M. oryzae* strain was selected based on its high CK production and high tolerance to drought stress as tested *in vitro*. To investigate the ability of *Methylobacterium* to alleviate drought stress, we analysed plant growth characteristics (shoot size, fresh and dry weight), physiological traits (photosynthesis, transpiration, Water Use Efficiency), biochemical parameters (Electrolyte Content, Electrolyte Leakage, chlorophyll content), plant water management (Leaf Water Content, Relative Water Level) and yield associated traits (number and mass of seeds, Harvest Index). The presence of *Methylobacterium* significantly improved the performance of plants exposed to drought by stimulating early growth of shoots and roots (32% and 51% increase, respectively), increasing photosynthetic rates (4.99 $\mu molCO_2/m^2/s$ for inoculated and 2.68 $\mu molCO_2/m^2/s$ for controls after 3 weeks of drought), and Harvest Index (5-fold over the non-inoculated lentils). We attribute the stress mitigating effect of *M. oryzae* to the bacterial CK delivered to plants. This conclusion was supported by the elevated levels of total CK pool detected by HPLC-MS/MS in the leaves of drought stressed, inoculated plants - 279.5pmol/gFW, compared to 62.1 pmol/gFW in non-inoculated individuals.

P-06-07

Recycled media affects growth and causes changes in hormone profiles in a microalgae

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Cell growth mechanism has been a focus of numerous cell biology studies. Increasing the efficiency of growth and product yield are of interest not just to academia, but to industry as well. Rising in popularity is the use of microalgae in industry. It is possible to manipulate and track parameters pertaining to essential growth. The capacity of microalgae to grow using recycled materials could be carried out through industry collaboration. We have begun investigating the use of recycled materials with primary interest in the function of cytokinins (CK) -- a cell growth modifying phytohormone -- and its influence on biomass accumulation paired with monitoring of trace carbon sources, pH, glucose consumption, supernatant depletion, cell count, morphology, and gene expression, under organic and recycled conditions. Previous work with microalgae indicates that CKs act as growth regulators; however, little is known about their explicit roles among microalgal species. We seek to understand the role of CK in the complex mechanisms underlying microalgal growth, which may affect culture productivity and industrial waste footprint, by using microalgae recycled materials as a source for sustainable nutritious supplements. This research seeks to unlock the full potential of microalgae natural growth yield while expand upon the roles of CKs in algal growth.

P-06-08

Influence of high temperatures on seed development of *Brassica napus* cultivars

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Crop production is dramatically affected by environmental conditions. In past decades, the average annual temperatures have arisen worldwide and current climate models predict temperature increase of 0,2 °C per decade. In crops such as maize and rice high temperatures might cause a decline by 5 to 17% of average grain yield for every 1oC temperature rise. High temperatures affect not only overall morphology of plant but also development and viability of both female and male gametes, which partly results in heat-induced sterility. Our research aims to understand how high temperatures would affect seed development in Brassica napus (rapeseed), an oilseed crop widely cultivated in Europe. Our study includes an analysis of the morphological changes in yield traits such as silique length, seed number, embryo development and number of branches in three *Brassica napus* cultivars (DH12075, Westar, Topas DH4079) in three different day temperatures (22 °C, 28 °C, 34 °C). Alterations of seed size and embryo morphology was analyzed daily over a period of 14 days after pollination. Most importantly yield traits (number of seeds, weight of 1000 seeds, flowering time, number of siliques on the main stem) was evaluated.

In response to high temperatures plants adapt their developmental program, notably by altering their hormone levels and signaling. And further analyses are in progress to identify changes in the hormonal response to high temperatures during early seed development in *Brassica napus*.

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P-06-09

Electronic noses detect volatile signatures of responses to auxin herbicides

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The use of gas analysis technologies to detect volatile organic compounds is becoming increasingly adopted in various sectors of precision agriculture. The latest generation of instrument offer "out of the lab" capability combined with high sensitivity. These instruments can be used either to detect a single chemical or a pattern/fingerprint of chemicals produced by a biological system. In this study, we have used GC-IMS (Gas Chromatography – Ion Mobility Spectrometer) to record the volatile response to the challenge of Arabidopsis (and other plants) to auxin herbicides. We find characteristic auxin-dependent volatile peaks and have recorded time- and dose-dependencies of the responses. Furthermore, we have found that the responses can be detected from single leaves within an hour of treatment. Herbicide-resistant lines do not respond, or respond considerably less than susceptible lines, indicating that the volatile signatures map to the auxin response. We will discuss the potential of this technology to provide rapid, straightforward diagnosis of auxin herbicide resistance.

P-06-10

Assessing the implications of cytokinins for mammalian cells

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The plant hormone cytokinin has long been known to modulate the dynamics of plant growth and development. However, reports on the impact of cytokinin on animal cells gained much attention in recent years. We thoroughly analyzed the impact of kinetin in cultured cells at various concentrations. We meticulously examined protective effects of cytokinin against

cytotoxic and genotoxic cellular conditions. By using the Ferric Reducing Antioxidant Power assay, we assessed the intrinsic antioxidant activity of kinetin in a cell-free system and in cells by using dihydroethidium staining method. We looked at kinetin effects in mammalian cells such as HL60 cells, HaCaT human keratinocyte cells, NRK rat epithelial kidney cells and human peripheral lymphocytes after monitoring cell viability. Kinetin manifests no antioxidant activity in the cell free system and high doses of kinetin (500 nM and higher) reduce cell viability and mediate DNA damage in vitro. On the other hand, low doses of kinetin confer better protection in cells against oxidative stress. Moreover, we also show that pretreatment of the cells with kinetin significantly reduces NQO-mediated reactive oxygen species production. Also, we showed that cytokinin retains cellular GSH levels when they were treated with the GSH-depleting agents. We will discuss the interaction between cytokinins and human cellular pathway targets to intrigue the plant community about the novel implications of cytokinin in mammalian cellular systems.

P-06-11

Short term salinity response of selected Brassica crops

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Soil salinity is one of the world wide problems affecting crop productivity. In order to evaluate early plant response to high salinity and characterise tolerance markers, three important Brassica crops: Chinese cabbage, white cabbage and kale were exposed to short term salt stress (24 h) in the range of NaCl concentrations (50, 100 and 200 mM). Physiological markers including Na^+/K^+ ratio and photosynthesis efficiency followed by biochemical stress markers such as proline, antioxidant enzymes and lipid peroxidation (MDA) as well as phytohormones

(ABA, SA, JA, JA-Ile, brassinosteroids and IAA) were measured. Significant increase in proline and MDA contents, which are usually reported as strong biomarkers of abiotic stress, were obtained in roots and leaves tissue of Brassica crops, although photosynthetic efficiency was reduced only in Chinese cabbage. In parallel, osmoregulation through increased levels of ABA was shown to be one of the most dominant markers of salinity response among Brassicas. Contrary, levels of jasmonates (JA and JA-Ile) were significantly lower upon high salt in roots and leaves of cultivars. Salinity affected accumulation of growth promoting hormones brassinosteroids and IAA. Levels of brasinolide where significantly increased in roots and leaves of Chinese cabbage compared to other two Brassica crops and similar pattern was observed for active IAA. Salinity induced successive increase in IAA precursors (TRP, IPyA, IAM and IAN) in roots and leaves of three Brassicas but levels of active IAA were significantly the highest in leaves of Chinese cabbage followed by increased level of catabolic oxIAA. Performed PCA analysis showed correlations among observed phytohormones, biochemical and physiological markers, and revealed Chinese cabbage as the most sensitive cultivar.

P-06-12

Low light mitigate cold stress response of *Arabidopsis*

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Light intensity, as well as light quality, can modify plant cold stress responses. *Arabidopsis thaliana* response to 5°C was compared at normal (150 µmol/m^2/s) or low light (20 µmol/m^2/s) intensity. In order to assess the role of light signaling pathways in cold and frost acclimation, cold responses of mutants in blue light receptors cryptochromes (*cry1*, *cry2*) or in red light receptors phytochromes (*phyA*, *phyB*) were compared with that of wt at both light intensities. Samples were collected 30 min, 6 h or 7 d of treatments.

Plants *phyB* showed the most changed phenotype characterised by small blades with long petioles. Cold stress slowed down the growth of all genotypes at both light intensities.

Temperature drop to 5°C resulted in fast (after 6 h) decrease of maximum quantum yield (QYmax), most profound in wt and *cry1*. The lowest non-photochemical quenching (NPQ) was found in *phyB*. Low light imposed decrease of QYmax after 7 d, most strongly in *cry2* and *phyA*. Combined stress significantly reduced stress impact on QYmax and steady-state quantum yield. The lowest NPQ value was detected in *cry1*. Low light had a short-term negative effect on membrane stability (mainly in *cry2*, *phyA* and *phyB*). Cold stress caused decrease in membrane stability in all tested genotypes after 7 d. Combination of both stresses had no significant impact on membrane stability. Cold had generally negligible effect on lipid peroxidation (MDA). Low light increased MDA levels in all genotypes after 30 min and 6 h (except *cry1*), stress combination had no additive effect. Plants *cry1* and *phyA* were more susceptible to frost than wt. Cold acclimation increased frost tolerance of all genotypes, but its positive effect was reduced by low light.
Distinct effects of stress treatments observed in the individual genotypes suggest an involvement of particular photoreceptors in cold and low light responses.
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P-06-13

Root engineering in barley and maize causes mineral enrichment in leaves and seeds and enhanced drought tolerance

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The size and architecture of the root system is an important trait of crop plants as it determines the access to water and soil nutrients. The plant hormone cytokinin is a negative regulator of

root growth and branching. To explore the potential of cytokinin modulations in improving root functions, we have generated transgenic barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) plants with an enlarged root system by enhancing cytokinin degradation specifically in roots. This was achieved through root-specific expression of a *CYTOKININ OXIDASE/DEHYDROGENASE* (*CKX*) gene. Enhanced biomass allocation to roots did not cause a penalty on shoot growth or seed yield indicating that these plants were not source-limited. In leaves of transgenic barley and maize lines, the concentration of several macro- and micro-elements was increased, in particular of those with low soil mobility (P, Mn, Zn). Importantly, barley seeds contained up to 44% more Zn, which is beneficial for human nutrition. A dampened stress response of transgenic barley lines to long-term drought conditions documented their lower drought sensitivity. Taken together, this work demonstrates that root engineering of cereals is a promising strategy to improve nutrient efficiency, bio-fortification and drought tolerance.

P-06-14

Comparison of intracellular trafficking pathways of auxin carrier depending on light growth conditions of *Arabidopsis thaliana* root

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The sessile life style of plants forces them to adapt constantly to changing environmental conditions. Auxin modulates plant architecture during development and adaptation processes, in a concentration-dependent manner, whereby auxin gradients are generated by active and polar cell-to-cell auxin transport through plasma membrane located auxin import and export carriers. Tightly controlled protein turnover guarantees that proteins are build up or removed at the right time, at the required place and in appropriate quantity. Individual auxin carrier show different trafficking responses, in dependence of external influences. The intracellular localization of the auxin carrier PIN2 alters upon changing light growth conditions of the root, and therefore we used it as our main model protein to study different protein turnover dynamics depending on light exposure to the root. In comparison to PIN2 we tested the trafficking behavior of other auxin carriers and their influence on root adaptation processes to changing light exposure. During standard lab growth conditions, the root is exposed constitutively to light, which results in different auxin carrier trafficking behavior compared to roots that were grown in darkness. Therefore, we applied external stresses, as gravitropic stimulus together with a chemical screen to track differences in endocytosis/trafficking processes of diverse auxin carrier in cross-talk with changing light growth conditions of the root.

P-06-15

Genome-wide transcriptomic analysis of BR-deficient Micro-Tom reveals correlations between drought stress tolerance and Brassinosteroid signaling in tomato

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Brassinosteroids (BRs) are plant steroid hormones that play crucial roles in a range of growth and developmental processes. Although BR signal transduction and biosynthetic pathways have been well characterized in model plants, their biological roles in an important crop, tomato (*Solanum lycopersicum*), remain unknown. Here, cultivated tomato (WT) and a BR synthesis mutant, Micro-Tom (MT), were compared using physiological and transcriptomic approaches. The cultivated tomato showed higher tolerance to drought and osmotic stresses than the MT tomato. However, BR-defective phenotypes of MT, including plant growth and stomatal closure defects, were completely recovered by application of exogenous BR or complementation with a *SlDWARF* gene. Using genome-wide transcriptome analysis, 619significantly differentially expressed genes (DEGs) were identified between WT and MT plants. Several DEGs were linked to known signaling networks, including those related to biotic/abiotic stress responses, lignification, cell wall development, and hormone responses. Consistent with the higher susceptibility of MT to drought stress, several gene sets involved in responses to drought and osmotic stress were differentially regulated between the WT and MT tomato plants. Our data suggest that BR signaling pathways are involved in mediating the response to abiotic stress via fine-tuning of abiotic stress-related gene networks in tomato plants.

P-06-16

The expression and function of Oryza sativa pseudo-histidine phosphotransfer protein 3 in response to light

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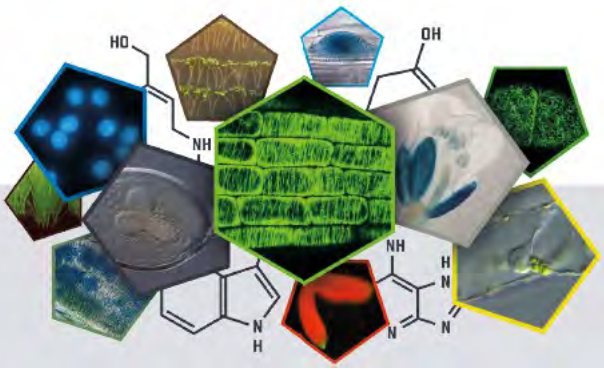
Two component system has been shown to involve in response to different environmental cues including light. Three pseudo-histidine phosphotransfer proteins (PHPs), which is critical for phosphoryl group transfer from the receptor kinase to response regulators, have been identified in rice. The conserved phosphorylation residue does not present in PHPs, which proposed as negative regulators in the cytokinin two-component system. One of the PHPs, OsPHP3, in rice is highly expressed at root caps. We detected two major alternative splicing mRNA of OsPHP3 and determined the expression in response to different environmental stimuli. The ratio of two alternative splicing PHP3 variants are significant altered in response to light signal. In addition, the function of OsPHP3 in the root also had been addressed and discussed. Taken together, these results indicate a possible function of OsPHP3 isoforms and the roles in rice lateral root growth.



P-06-17

Hormonal dynamics in cold and frost stress responses in monocots

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The necessary prerequisite for plant survival in temperate zones is their ability to cope with cold and frost stresses. Plant interactions with environment are controlled by plant hormones. Hormonal dynamics differ in the individual phases of stress response. Cold shock (drop from 20°C to 4°C) was associated with transient stimulation of the level of abscisic acid (ABA) in *Triticum aestivum* crowns and leaves. Fast down-regulation of cytokinins (CKs), gibberellins and auxins in leaves, later on in crowns (especially in winter variety) led to the growth inhibition. The acclimation phase was accompanied by CK, gibberellin and auxin elevation, together with up-regulation of salicylic acid (SA) and jasmonic acid (JA). Hormonal changes associated with sub-zero stress were studied in 6 *Lolium perenne* clones, which differed in their frost tolerance. Cold acclimation (3°C for 7 days) led to elevation of active CKs in leaves and crowns. Subsequent frost treatment (-7°C for 4 days) resulted in CK and auxin increase in roots and leaves, but decrease in crowns. Direct frost treatment (without cold acclimation) resulted in suppression of CKs and auxins in roots and leaves, but their elevation in crowns, indicating their preferential protection. Cold acclimation was accompanied with ABA and SA increase in whole plants. Subsequent frost led to ABA and SA down-regulation in crowns and leaves. Direct frost treatment resulted in ABA and SA elevation. JA content correlated with cold tolerance under

control conditions. During acclimation, JA content decreased in roots and crowns, while increased in leaves. Subsequent frost exposure reverted JA changes in the corresponding tissues. Direct frost application had similar, even if stronger effect like acclimation, especially in leaves. The obtained results show specific effects of cold acclimation as well as complex cross-talk among hormones.

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Searching for functions of cytokinins in the streptophyte alga *Klebsormidium nitens*

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The colonization of land by early divergent streptophytes required major adaptations in the physiology and molecular mechanisms in order to cope with environmental challenges like UV radiation or osmotic stress. Hormone regulatory mechanisms are very likely to have contributed to the evolution of stress tolerance. We addressed the question, whether cytokinins may play a role in the regulation of tolerance towards osmotic stress in the alga *Klebsormidium nitens* (Klebsormidiophyceae).
Using bioinformatic tools we screened the *K. nitens* genome (Hori et al., Nature Comm, 5, 3978, 2014) for orthologs of the cytokinin machinery. We also checked whether *K. nitens* is capable of depleting the culture medium from externally supplied isopentenyladenine and benzyladenine by a HPLC-based method. Experiments in which cultures of *K. nitens* were simultaneously treated with mannitol and iP indicated that the cytokinin regulatory network might play a role in the tuning of osmotolerance. Quantitative real time PCR data for expression of cytokinin related genes revealed that osmostress can affect the cytokinin mediated gene regulation.

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