ACPD 2023

Auxins and Cytokinins in Plant Development 2023

... and cross-talk with other phytohormones in interactions with the changing environment

International Symposium 2023

June 25–29, 2023 | Prague, Czech Republic

BOOK OF ABSTRACTS

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ORAL PRESENTATIONS

Symposium Opening Lectures

O-01

Hormonal regulation of root development: auxin and cytokinin cross-talk and beyond

Eva Benková

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Auxin and cytokinin are key hormonal orchestrators of root system architecture and its developmental plasticity. In the past, we have identified several convergence points and pathways that might integrate auxin and cytokinin hormonal inputs to coordinate root organ development. Intriguingly, some of these recently identified molecular components seem to exceed their simple function in the auxin-cytokinin cross-talk, and they provide functional links with other regulatory pathways, for instance, by a mediating perception of environmental stimuli, such as abiotic stress, nitrate availability, or by controlling the sub-cellular trafficking. Our insights into mechanisms integrating these auxin and cytokinin regulatory path-ways into complex molecular networks that coordinate plant growth and its flexibility to varying external inputs will be discussed.

O-02

Root developmental plasticity: challenges and new insights

Joseph G. Dubrovsky

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Roots are important for plant adaptation to ever changing environment and represent a significant sink of Carbon on Earth. Understanding root biology, growth, and developmental plasticity is important for sustained food production. To maintain developmental plasticity, many different mechanisms have evolved. Therefore, a challenging task is the comprehension of how a parent root axis growth is coordinated with new root production. I will discuss how the root axis growth in Angiosperms can be modulated, what are the limits in cell cycle duration in the root apical meristem (RAM), how indeterminate root growth is maintained and how it can be abolished. Indeterminacy-to-determinacy switch is one of the mechanisms modulating root system architecture and root growth. I will give examples of constitutive determinate root growth and present new insights on metabolic mechanisms involved in the RAM maintenance that apparently are independent of classical regulatory pathways. I will also overview different strategies of lateral root (LR) initiation, taking place from embryogenesis to post-germination and from the RAM to differentiation zones. Understanding plasticity in LR founder cell specification and variability in founder cell number, even within the same species, represents a challenge for comprehension of root development. I will discuss also to which extent LR founder cell recruitment is a part of developmental plasticity. Finally, I will discuss the role of parent root tissues, specifically procambial cells, in the formation of vascular bridge between the parent and LR and how auxin is involved in this and other processes.

DGAPA-UNAM IN204221 support is acknowledged.

Biosynthesis and metabolism

Session to commemorate the legacy of Mirek Kamínek

Session Opening Lecture

O-03

Exploration of novel genes involved in cytokinin and auxin metabolisms using natural variations in rice

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Cytokinins and auxins play a central role in the regulation of plant growth and development. In the past two decades, main pathways of their biosynthesis and metabolism have been elucidated. In cytokinins, the central activation pathway is the removal of ribose phosphate from the ribotide precursors catalyzed by LOG, but cytokinins also occur as riboside precursors, such as trans-zeatin riboside, which are known to be the dominant form of root-to-shoot translocation via xylem. However, little is known about enzymes involved in the riboside precursor metabolism. On the other hand, major inactivation pathway of indole-3-acetic acid is the conjugation with amino acid catalyzed by GH3 and the oxidation of the conjugates catalyzed by DAO. In our research project, potentially useful naturally mutated loci affecting phytohormone concentration in rice (*Oryza sativa*) were searched by LC/MS-based phytohormone profiling using chromosome segment substitution lines. As a result, natural mutant loci affecting the level of cytokinin riboside precursor, was detected on chromosome 5 and affecting that of IA-amino acid was detected on chromosome 6. I will show you the causal genes identified and the physiological role in the two phytohormones' action.

O-04

Control of cytokinin responses by endoplasmic reticulum-associated degradation of CKX proteins

Lisa Theisl, Isabel Bartrina, Tomas Werner

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Eukaryotic organisms possess quality-control mechanisms that monitor protein folding in the endoplasmic reticulum (ER) and eliminate non-native proteins through ER-associated degradation (ERAD). Although it is widely acknowledged that this evolutionary conserved process is critical for maintaining protein homeostasis in the ER, only little is known about how the ERAD pathway is linked to different physiological and developmental processes in plants. It has recently been shown that cytokinin-degrading CKX proteins are subjected to ERAD, but the underlying mechanisms have remained largely unknown. Here we present our progress in identifying the molecular components involved in the CKX ERAD and discuss their function in tuning cytokinin responses during plant development.

O-05

Amino acid conjugation of oxIAA is a secondary metabolic regulation involved in auxin homeostasis

Federica Brunoni[1], Aleš Pěnčík[1], Asta Žukauskaitė[2], Anita Ament[1], Martina Kopečná[3], Silvio Collani[4], David Kopečný[3], Ondřej Novák[1]

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Auxin inactivation plays a crucial role in auxin homeostasis and metabolism. The mechanisms by which auxin-inactivating enzymes governed auxin metabolism remained fragmented and these enzymes appeared to participate in different catabolic pathways. Recent discoveries placed the IAA-inactivating enzymes GH3, ILR1, and DAO into a single catabolic route. According to this model, IAA is mainly inactivated through conjugation with amino acids by GH3 enzymes. DAO functions as an oxidase of IAA-aa conjugates to produce oxIAA-aa conjugates downstream of GH3, and oxIAA is produced from oxIAA-aa hydrolysis by ILR1. This model was proposed to coordinately regulate IAA recycling and degradation in Arabidopsis and monocots. We further investigated the involvement of these players and found that an additional pathway contributes to the modulation of auxin homeostasis. We collected data demonstrating that oxIAA is also a substrate for GH3 enzymes. Thus, oxIAA-aa conjugates can also be formed by GH3-mediated conjugation of oxIAA. Functional analysis of Arabidopsis GH3s revealed that only the group of IAA-conjugating GH3s accepts oxIAA as a substrate and that oxIAA conjugation is a slower reaction than IAA conjugation. Inspection of these pathways in evolutionarily distant species revealed that GH3-mediated oxIAA conjugate formation contributes only to a minor extent to the formation of oxIAA-aa conjugates in Arabidopsis and moss. On the contrary, oxIAA amino acid conjugation is the main pathway for oxIAA-aa formation in spruce. Our results suggest that GH3-mediated oxIAA conjugation is a metabolic pathway that occurred early during land plant evolution, and its contribution to IAA homeostasis is species-dependent.

O-06

Mass spectrometric screening of IAA metabolism in cultured cells and plants; detection of the auxin decarboxylation pathway

Petre I. Dobrev, Roberta Filepová, Jozef Lacek, Zuzana Vondráková, Karel Müller, Petr Maršík, Lenka Drašarová, Petr Hošek, Jan Petrášek

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The natural plant hormone auxin indole-3-acetic acid (IAA) influences many physiological processes in plants. Plants respond to minute concentrations of IAA, suggesting that it must be tightly controlled at the levels of biosynthesis, metabolism and transport. Here, the metabolism of IAA was studied in detail using tobacco BY-2 cell suspensions as a model. A combination of labeled/unlabeled substrate feeding, global untargeted mass spectrometric (MS) scanning and selective MS filtering allowed the detection of 17 auxin metabolites, 15 of which were identified. A study of intermediate metabolism and dynamics revealed eight major pathways: three amino acid conjugation pathways with aspartate, glutamate, and glutamine followed by their 2-oxidation with the enzyme DAO; side-chain glucosyl ester formation; direct 2-oxidation; two decarboxylation pathways; and a pathway producing an unidentified metabolite. We found that the majority of the detected auxin metabolites occur naturally in several plant species, suggesting that the auxin metabolic pathways observed in the BY-2 cell suspension also occur *in planta*. Our finding that the IAA decarboxylation pathway occurs *in planta*, and the previous reports of auxin activity of some metabolites of this pathway, suggest that at least some of the biological effects of IAA may be explained by its conversion to decarboxylative metabolites. However, this requires further in-depth investigation.

Supported by The European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/ 0.0/0.0/16_019/0000738).

O-07

Disclosing GH3-mediated inactivation mechanisms that govern auxin homeostasis in plants

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The GH3 family of amido synthetases catalyzes the ATP-dependent conjugation of acidic phytohormones, such as JA, IAA, and SA, with amino acids, producing either active or inactive forms of these hormones. In the context of auxin homeostasis, GH3 members mediate the first inactivation step by forming IAA-aa. Then IAA-aa can be converted back to IAA *via* IAA-aa hydrolases (ILR/ILL) or oxidized to oxIAA-aa (DAO). These reactions are described to be spatially separated on subcellular scales, with the IAA conjugation and oxidation steps occurring in the cytosol, while IAA-aa hydrolysis in the endoplasmic reticulum. Although IAA-aa are expected to be transported from cytosol to ER, this was not demonstrated yet. Our *in silico* prediction reveals a strong binding of IAA-aa to the inward-facing positions of the ER-localized IAA transporters, PIN5 and PIN8, indicating IAA-aa transport in/out of the ER. Recent evidence suggests that the nucleus may also be involved in auxin homeostasis. DAO was reported to have nuclear and cytoplasmic localization, and specific IAA metabolites were detected in sorted nuclei. We have generated *GH3-GFP* Arabidopsis transgenic lines and detected GH3 enzymes not only in the cytosol, as previously reported, but also in the nucleus, supporting a direct role for the nucleus in auxin metabolism.

Supported by grant no. DSGC-2021-0171 under OPIE project no. CZ.02.2.69/0.0/0.0/19_073/0016713.

Biosynthesis and metabolism

Session Opening Lecture

O-08

Metabolic regulation of two natural auxins in plants

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Auxin is a key regulator of various aspects of plant growth and development. Plants produce the main auxin indole-3-acetic acid (IAA) through the indole-3-pyruvate pathway. It has recently been demonstrated that IAA is mainly inactivated via the GH3-ILR1-DAO pathway in seed-producing plants. However, IAA inactivation processes remain elusive in spore-producing plants. We reinvestigated the IAA inactivation pathway in lycophytes, hornworts, liverworts and mosses by LC-MS/MS analysis of auxin metabolites. We detected the specific metabolites of the GH3-ILR1-DAO pathway in all plants we analyzed, except for the moss *Marchantia polymorpha*, suggesting that the GH3-ILR1-DAO IAA inactivation pathway is conserved in both seed- and spore-producing plants. In addition to IAA, plants synthesize phenylacetic acid (PAA), which exhibits less biological activity than IAA, as a natural auxin. Although IAA and PAA can induce similar auxin responsive genes via the TIR1/AFB pathway, a physiological role of PAA is still largely unknown. We studied the metabolic regulation of IAA and PAA in Arabidopsis. Interestingly, the amounts of PAA and its metabolites were reduced in elongated stems under high temperature conditions, while the level of IAA was elevated, suggesting that PAA may play a role in thermomorphogenesis.

O-09

How balancing auxin homeostasis and transport shapes plants

Ute Voss

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Since colonizing land, plants have developed a wide variety of mechanisms to tolerate a broad range of abiotic stresses that include flooding, drought, extreme temperatures, high salinity, or nutrient limitation. Roots play a key role acclimating plants to these stresses, as their developmental plasticity enables them to grow towards more favourable conditions and away from limiting or harmful stresses.

The phytohormone auxin plays a key role translating these environmental signals into developmental outputs. This is achieved by modulating auxin levels and/or signalling. Auxin gradients are established and maintained by a tightly regulated interplay between homeostasis, signalling, and transport. Cellular auxin levels are also regulated depending on tissue or environmental contexts and these result in specific developmental outputs, such as enhancing or repressing root branching, modulating (lateral) root angle. While recent advances have shed light on the biochemistry of the auxin degradation pathway, it is not well understood how auxin degrading enzymes, in particular the DAOs are regulated and thereby contribute to plant development and acclimatisation to abiotic stresses or how this regulation evolved.

O-10

New generation of CKX inhibitors: review and applications

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Recently we have developed three chemical groups of compounds derived from diphenyl urea with various inhibitory activities against cytokinin oxidase/dehydrogenase (CKX). These compounds protect the degradation of cytokinins in plants, thus enhancing their content. This effect can be used for research and biotechnological applications. Each group contains CKX inhibitors with nanomolar IC_{50} values obtained with maize ZmCKX1 and/or Arabidopsis AtCKX2. The binding mode of the most active compounds to maize ZmCKX4 and ZmCKX8 was characterized using high-resolution crystal complex structures. The CKX inhibitors were used successfully in plant tissue culture techniques to improve shoot regeneration or to induce direct somatic embryogenesis. Our data further indicate that CKX inhibitors can improve plant stress tolerance, nutrient use efficiency, production of amino acids as well as of some special metabolites, and finally the seed yield.

Novel Methods and Techniques

Session Opening Lecture

O-11

Plant Hormonomics: Phytohormone metabolite profiling

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The identification and quantification of plant hormones in plant tissues are necessary for physiological studies of their metabolism and mode of action. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. A fast chromatography technique, the ultra-high performance liquid chromatography (UHPLC) was coupled to triple quadrupole mass spectrometer equipped with an electrospray interface (ESI-MS/MS). In selective multi reaction monitoring (MRM) mode, the detection limit for most of phytohormones (cytokinins, auxins, abscisic acid, jasmonates, gibberellins, brassinosteroids) was close to 1 fmol and achieved linear range was at least five orders of magnitude. Use of our procedures can allow the quantification of plant hormones and their derivatives (in total around 150 compounds) in very limited amounts of plant material. Moreover, we also focus on tissue- and cell-specific analyses of phytohormones, combining novel approaches such as a simple one-step purification protocol based on in-tip micro solid-phase extraction and a class-specific miniaturized immunoaffinity chromatography method. The methods provide substantial improvements in terms of robustness, sensitivity, selectivity, convenience, throughput and cost-effectiveness over previous methods published. The new and modern analytical approaches make possible a new direction in plant hormone metabolite profiling. We believe that UHPLC-ESI-MS/MS technology can be used for fast and sensitive quantitative analysis showing reproducibility in the plant hormone profiling in different plant tissue and cell extracts.

O-12

Multi-class plant hormone analysis using hydrophilic interaction chromatography-tandem mass spectrometry

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Quantitative analysis of plant hormones is an integral part of the studies of plant development. All currently used methods are based on reversed-phase liquid chromatography hyphenated with mass spectrometry. While mass spectrometry will not be replaced in a near future by another detection, liquid chromatography offers alternatives to reversed-phase system. We believe that these alternatives are not as fully explored in the field of plant hormone research. For this reason, we developed a novel method based on hydrophilic interaction chromatography (HILIC). The method enables the analysis of 50 plant hormones including ACC, CKs, AUX, ABAs, JAs, and SAs. It offers higher sensitivity and stronger retention of polar compounds without the need for derivatization. Sample pre-treatment is based on micro-SPE using RP packing material that brings orthogonality into the entire method. Fundamental features of HILIC-MS/MS, its potential in the analysis of plant hormones and other bioactive compounds, as well as general analytical parameters (selectivity, sensitivity, detection limits, recovery, robustness) will be discussed.

O-13

Accurate in silico assessment of the function of molecules and their targets

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The method of feedback of model and laboratory facts is the main objective. The detailed study of molecular structures at the 3D level has led to two main directions: the analysis of compounds in terms of their biological effects and the search for potential protein targets.

As an example, consider first the discovery of new active auxin molecules occupied by different atoms. Brominated phenolic compounds at positions 2 and 6 with random substitutions at positions 3, 4, and 5, as well as other chemicals known to have auxin-like activity or to be inactive, have been studied. Their molecular structure, biological activity, transport ability, and signaling pathways were studied. Quantum molecular similarity analysis was performed to categorize the molecular structures and select candidates for study. For the selected candidates, binding affinities were assessed with Tir 1 Fbox at the DFT level. After completion of the transport, signaling, and physiological experiments, the necessary statistical studies were performed to determine the effects of each compound. Finally, a structure-function analysis was performed.

Subsequently, some proposed interactions between CUPINs and auxin are discussed. Classification of auxin-like ligands according to physiological functions and chemical structures, screening of binding energies of auxin-like ligands with putative binding pockets, and finally selection of sequences of CUPIN proteins with better prediction of auxin binding. It opens the analysis of CUPIN proteins most likely to bind auxin-like molecules.

O-14

Alleviating the barrier of adventitious roots formation in recalcitrant mature tissue by slow release of a synthetic auxin

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Clonal propagation of plants by induction of adventitious roots (ARs) from stem cuttings is a requisite step in breeding programs. Nevertheless, a major barrier exists for propagating valuable plants that naturally have low capacity to form ARs. Due to the central role of auxin in organogenesis, indole-3-butyric acid (IBA) is often utilized, yet many recalcitrant plants do not form ARs in response to such treatment. We describe the synthesis and screening of a focused library of synthetic auxin conjugates in *Eucalyptus grandis* cuttings, highlighting 4-chlorophenoxyacetic acid-L-tryptophan-OMe as a competent enhancer of adventitious rooting in a number of recalcitrant woody plants. Comprehensive metabolic and functional analyses revealed that this activity is engendered by prolonged auxin signaling due to initial fast uptake and slow release and clearance of the free auxin 4-chlorophenoxyacetic acid. This work highlights the utility of a slow-release strategy for bioactive compounds and provides an exemplar for further rational development of more effective plant-growth regulators for agriculture.

Transport

Session Opening Lecture

O-15

A biochemical view on auxin and cytokinin transporters

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Both auxins and cytokinins are signaling molecules likewise essential for proper plant development and performance. Plants move auxins and cytokinins over short and long distances resulting in hormone maxima, gradients, and cellular and subcellular sinks.

In a first part of my talk, I will summarize the current knowledge of auxins and cytokinin transporters in respect to their biochemical activities. I will try to answer the long-lasting question why plants need that many auxins and cytokinin transporters and especially why there seems to exist an evolutionary need to employ different modes of transporter directionalities and energization. It appears that auxin (and eventually also cytokinin transporters) might be part of a “hormone homeostat” system in analogy to nutrient cycling.

In a second part, I will give an update on the regulation and evolution of ABCB-type auxin exporters. So far, all characterized Auxin Transporting ABCBs (so-called ATAs) contain an evolutionary conserved D/E-P motif that seems to be refolded by PPIases, leading to a transient activation of auxin export activity. Using site-directed mutagenesis of key residues on ABCB1 and its interacting PPIase, TWISTED DWARF1, we provide supportive evidence for such a novel mode of transient transporter regulation.

O-16

Medicago truncatula ABCG40 is a cytokinin importer negatively regulating lateral root density and nodule number

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Plant roots show high plasticity to meet needs during fluctuations of nitrogen availability in the soil and can adapt both their physiology and morphology, accordingly. Numerous studies suggest a relevant role of cytokinin (CK) distribution in shaping of root systems. Nonetheless, our knowledge about an involvement of short-distance CK translocation in root mineral nutrition is still scarce and specific role of CK transporters in root morphology has yet to be established. We identified and characterized the *Medicago truncatula* full-size ATP-binding cassette (ABC) transporter belonging to the G subfamily, namely MtABCG40 as a CK importer. Its expression is root-specific and is induced by nitrogen deprivation and CKs. Our analyses indicate that MtABCG40 has a negative impact on lateral root density through decreased lateral root initiation and enhancement of primary root elongation. The *mtabcg40* plants have an accelerated pace of cell division in the developing lateral root primordia and reduced the size of root apical meristem (RAM). The MtABCG40 action affects CK signaling and impacts the cellular auxin content. Moreover, in line with postulated resemblance to lateral roots, we also observed an inhibitory influence of this transporter on nodule number. We present data that demonstrate a full-size ABCG transporter with a novel function in legumes and CK transport.

This work was supported by the Polish National Science Centre (Grants No. 2015/19/B/NZ9/03548)

O-17

Deciphering the functions of purine permeases in cytokinin transport

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Plants regulate cytokinin distribution through metabolic reactions and transport processes occurring at biological membranes. Previous research has identified four members of the Arabidopsis *PURINE PERMEASE* (*PUP*) family as non-specific cytokinin transporters. With 22 *PUPs* present in Arabidopsis, our goal was to enhance our comprehension of this transporter family by examining the sub-cellular localization, transport capabilities, and biological roles of specific members. By using a complete set of transcriptional reporter lines, we provided a detailed characterization of *PUP* expression patterns, which suggest *PUP* involvement in numerous developmental processes in roots and shoots. Likewise, we identified four members (*PUP4*, *PUP6*, *PUP14*, and *PUP18*) likely to play a role in lateral root development and emergence. Despite confirming PUP14's ability to import adenine, and to a lesser extent, trans-zeatin, our overexpression and mutant lines challenged the suggested function of PUP14 as a cytokinin signaling repressor. Root development was undisturbed in *pup14* mutants, while *PUP14* overexpression did not alter the *TCSn::GFP* expression. We also characterized the single PUP protein encoded by the liverwort *Marchantia polymorpha*, which revealed a higher affinity for trans-zeatin in oocyte uptake assays. However, the *M. polymorpha pup* mutant displayed normal growth rate and development, suggesting that this protein, which localizes to the plasma membrane and endoplasmic reticulum, is not essential for cytokinin signaling. Finally, we discovered that *PUPs* likely evolved earlier than previously thought, dating their origin to the dawn of land plants. Together, these approaches have contributed to a better understanding of *PUP* gene functions, which extend beyond cytokinin transport.

O-18

Zooming on PIN structure through a membrane topology lens – is there something to see still?

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PIN proteins establish the auxin concentration gradient, which coordinates plant growth. PIN1-4 and 7 localized at the plasma membrane (PM) and facilitate polar auxin transport while the endoplasmic reticulum (ER) localized PIN5 and PIN8 maintain the intracellular auxin homeostasis. Although an antagonistic activity of PIN5 and PIN8 proteins in regulating the intracellular auxin homeostasis and other developmental events has been reported. But the structural basis how the two proteins, which localize at the same intracellular compartment, antagonize each other remains obscured. Combining immunolocalization, pH-dependent fluorescent quenching, and topology prediction programs, we map the membrane topology of PIN5 and PIN8 in *Arabidopsis thaliana* root cells. Our results hint at a divergent membrane topology of PIN5 and 8 that might reflect different and often mutually opposing activities of these intracellular auxin homeostasis regulators. Thus, using protein membrane topology determination techniques we look at the PINs from yet another angle that might reveal more still, than other structural methods.

O-19

HSP90 provides plasticity to auxin transport and plant development

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Heat shock protein 90 (HSP90) is an essential chaperone involved in the folding and maturation of many key proteins. The recruitment of HSP90 client proteins is controlled by co-chaperones, such as FK506 binding proteins (FKBPs), which are thought to bind HSP90 via their tetratricopeptide repeat (TPR) domains. Recently, the Arabidopsis FKBP42, TWISTED DWARF1, was shown to control the biogenesis and transport activity of interacting auxin transporters of the ABCB family.

Here, we identify a subset of cytosolic HSP90 isoforms as valid interactors of TWISTED DWARF1. By mutagenesis, we dissect the impact of FKBP and TPR domains on ABCB1 transport activity and biogenesis, respectively. We provide pharmacological and genetic evidence that only a subset of TWD1-interacting ABCBs – but not other auxin transporters – are HSP90 clients in plants, qualifying TWD1 as a co-chaperone of HSP90 action. We provide a strong correlation between the effects of HSP90 inhibition on ABCB-mediated development and ABCB plasma membrane stability on the one hand and ABCB cycling rate on the other.

Our results classify cytosolic HSP90s as root-specific, positive regulators of polar auxin transport that confer plasticity to ABCB-controlled auxin transport and plant development by stabilizing the plasma membrane presence of redundant ABCB isoforms.

Session Opening Lecture

O-20

Substrate recognition and transport mechanism of the PIN-FORMED auxin exporters

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The PIN-FORMED family of transporters plays a crucial role in the transport of auxin. The recently solved structures of three different PINs provide insight into substrate recognition and auxin transport in PINs. PINs exist as dimers, with each monomer comprising a transporter and a scaffold domain. The auxin binding cavity is divided into a vestibule and a binding chamber, and are mechanistically balanced by a support site connected to a 'dipole-neutralizing residue'. The binding chamber and the support site are linked by a crossover motif that serves as the pivot point for transport. Different conformations and binding configurations for auxins likely represent steps in an elevator transport mechanism know from other protein families. Substrate recognition by PINs will be discussed.

O-21

There's a new family in town: AZGs as cytokinin transporters

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The AZA-GUANINE RESISTANT (AZG) protein family plays an important role in plant development and morphogenesis. With a single ancestor in algae, the family has diversified in lycophytes and consist of two members, AZG1 and AZG2, in most sequenced angiosperms. Originally described as purine transporters, subsequent experiments in *Arabidopsis thaliana* have shown that AZG1 and AZG2 can transport cytokinins with high affinity.

Despite their high level of similarity, AZG1 and AZG2 differ in their transport mechanisms, and their niche in plant physiology is also different. AZG2 is mainly expressed in a small group of cells surrounding the lateral root primordium and accompanies the development of primordium during its developmental stages by modulating its emergence. AZG2 expression is induced by auxins via ARF7, linking the two major morphogenetic hormones. In contrast, AZG1 is a plasma membrane transporter expressed in most organs and stages of plant development. Together with AZG2, AZG1 is also involved in auxin-cytokinin cross-talk by interacting with PIN1 at the plasma membrane and preventing its degradation. In addition, the AZGs can form homo- and hetero-oligomers. The activity of AZG1 and AZG2 is required for proper cytokinin perception and their absence or overexpression affects the architecture of the root system and impairs the plant's ability to respond to the environment.

In summary, the AZGs are a novel family of cytokinin transporters that play a key role in modulating plant body shape. AZGs are of particular interest because they represent nodes for cross-regulation between auxin and cytokinin signaling.

O-22

D6PK plasma membrane polarity requires a repeated CXX(X)P motif and PDK1-dependent phosphorylation

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D6 PROTEIN KINASE (D6PK) is a polar localized plasma membrane associated kinase from *Arabidopsis thaliana* that activates polar distributed PIN-FORMED (PIN) auxin transporters. D6PK moves rapidly to and from the plasma membrane independent of its PIN targets. The middle D6PK domain, an insertion between kinase subdomains VII and VIII, is required and sufficient for association and polarity of the D6PK plasma membrane. How D6PK polarity is established and maintained remains to be shown. Here we show that cysteines from repeated middle domain CXX(X)P motifs are S-acylated and required for D6PK membrane association. While D6PK S-acylation is not detectably regulated during intracellular transport, phosphorylation of adjacent serine residues in dependence on the upstream 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE (PDKs) promotes D6PK transport, controls D6PK residence time at the plasma membrane, and prevents it its lateral diffusion. We thus identify new mechanisms for the regulation of plasma membrane interaction and polarity of D6PK.

Signalling

Session Opening Lecture

O-23

Cytokinin signaling regulates phase transitions and reproductive behavior

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Plants undergo during their lifetime several important developmental transitions, including seed germination, the juvenile-to-adult transition during vegetative growth (vegetative phase change, VPC) enabling the plant to respond to flowering-promoting cues, and the transition to flowering itself. We have analysed the role of cytokinin signaling during these developmental transitions in Arabidopsis thaliana. VPC is regulated by an age-dependent mechanism consisting of the opposite action of microRNAs miR156/miR157 and miR172, and their respective target genes belonging to the SPL and AP2-like families of transcriptional regulators. We discovered that cytokinin promotes VPC through this age pathway depending on the AHK2 and AHK3 receptors and distinct B-type ARRs, linking cytokinin action to miRNAs. The miR172 targets TOE1 and TOE2 were necessary and sufficient to mediate the influence of cytokinin on VPC. In recent work we have studied a possible crosstalk between cytokinin and gibberellin in regulating VPC. Likewise, gene expression and genetic studies identified a role for cytokinin signaling in promoting the transition to flowering, in particular under short day conditions and involving as well components of the age pathway. Reciprocal grafting experiments revealed that root-derived cytokinin is an important flowering signal. Furthermore, in reproductive tissues of Arabidopsis cytokinin regulates several traits impacting seed yield, including flower and silique number, ovule distance and the duration of flowering. I will report on our efforts to achieve yield enhancement in mono- and dicot crop plants, barley and oilseed rape, by modulating the cytokinin signal strength based on results obtained in Arabidopsis.

O-24

Decoy receptor fine-tunes cytokinin signaling in Arabidopsis

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The plant hormone cytokinin plays a vital role in plant growth and development. While the molecular mechanism of cytokinin signal perception and transduction is well described, regulating this cascade *in planta* seems more ambiguous. The Histidine kinase receptor CRE1 (CYTOKININ RESPONSE 1)/AHK4 (ARABIDOPSIS HISTIDINE KINASE 4)/WOL (WOODEN LEG), is the crucial element of the cytokinin signaling cascade in *Arabidopsis*. The cytokinin-signaling pathway is under stringent negative feedback control executed at several levels. We discovered a novel *CRE1* transcript variant with a negative feedback regulatory function. This transcript encodes for a truncated receptor lacking the receiver domain essential for activating the cascade. Our results demonstrate that the truncated receptor acts as a decoy competing for ligand binding with the canonical CRE1 receptors, ultimately attenuating cytokinin signaling. Thus, we propose a novel regulatory mechanism of cytokinin perception mediated by alternative splicing of CRE1 receptors.

O-25

Cytokinin N-conjugated Forms are Involved in Delaying Natural and Abiotic Stress Senescence

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Cytokinin (CK) is a well-studied plant hormone involved in regulating many important developmental and stress-related processes such as senescence. However, CK is often treated as a single compound even though many plants have around 30 different endogenous forms with varied levels of biological activity. CK base forms (tZ – trans-Zeatin, iP – isopentenyl-adenine, DHZ – Dihydro-Zeatin, and cZ – cis-Zeatin) are generally considered as “active” while highly abundant CK-N-glucoside (CKNG) forms, composed of a CK base irreversibly conjugated to a glucose molecule, have largely been thought of as “inactive” for many years. Our recent results have revealed “active” functional CK roles for several N7 and N9 glucoside forms after re-examination in classic CK-bioassays, such as in delaying dark induced leaf senescence. We have further examined the basic role of both CK base and CKNG forms through time course analyses of this classic bioassay by pairing physiology with transcriptomic measurements. This has revealed a suite of developmentally affected genes that occur during dark induced senescence that are countered to by CK base and CKNG forms to delay senescence. Some effects occur in similar manners, while other base and CKNG forms act uniquely. Further examinations of CK forms to delay abiotic salt stress senescence was also found to occur using similar paired methodology for tZ forms. Discussion of these findings in context of developmental and abiotic stress will be presented, but appear to further indicate an active role for CKNG in senescence responses.

O-26

Auxins as herbicides

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Auxins are used globally as selective herbicides. It is anticipated that herbicide action is an overload of binding sites in the Transport Inhibitor Response 1 (TIR1) family of auxin receptors but is this the whole picture? The auxin herbicides fall into five chemical classes and the receptors into three clades. We use purified receptor proteins to measure binding efficacy and have conducted quantitative structure activity relationship (qSAR) assays for Arabidopsis TIR1, AFB2 and AFB5 alongside qSAR data for biological efficacy. We have also evaluated binding specificity across species. The data highlight differences in receptor selectivity and some systematic differences between activities in vitro and in vivo. We then studied plant responses to auxin herbicide applications in barley and in Arabidopsis and mapped field resistance to auxin herbicides which highlights the importance of Aux/IAA co-receptors in the system. There is more than agonist overload to auxin herbicide action.

O-27

Designing novel synthetic cytokinin reporters based on scRNA-seq data

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During plant development, a precise balance of cytokinin is crucial for correct growth and patterning. However, it remains unclear how this is achieved across different cell types and in the context of a growing organ. To understand the tissue-specific responses of increased cytokinin levels, we profiled the transcriptional effect of a cytokinin treatment on root meristem cells at single-cell resolution. Clear transcriptional changes were observed for most cell types, while some subpopulations remained largely unaffected, suggesting tissue-specific responses. As expected, primary response genes of the cytokinin signaling pathway, such as A-type ARABIDOPSIS RESPONSE REGULATORS, were recovered as cytokinin inducible in all cell clusters. Interestingly, we found many cell type specific differentially expressed genes that were unique to tissues generally associated with cytokinin, as shown by the traditional reporter of cytokinin activity; the Two-Component-Signaling (TCS) reporter. Since the TCS reporter is based on a single DNA-binding motif, we opted to leverage our dataset for novel, cell type specific motifs and were able to identify several. Based on these predictions we are currently generating a set of new cytokinin reporters, both generic and cell type specific.

O-28

Deciphering the role of HIPP proteins in regulating auxin signaling responses

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The plant hormone auxin plays a central role in regulating plant growth and development. The auxin signaling pathway is centered around AUXIN RESPONSE FACTORS (ARFs), transcription factors that couple the perception of auxin hormone and changes in gene expression. Here we show that HEAVY METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEINS (HIPPs) interact with ARF transcription factors and are necessary for the transcriptional responses to auxin. HIPPs are plant-specific proteins of largely unknown physiological function. Our research revealed that cluster III HIPP proteins from Arabidopsis localize specifically to plasmodesmata and that they play important role in regulating a wide variety of plant developmental processes. The transcriptional profiling of *hipp* loss-of-function mutants identified extensive differences in the expression of auxin-associated genes. Protein-protein interactions studies revealed that cluster III HIPP proteins interact with various members of the ARF transcription family, suggesting that HIPP-ARF interactions are responsible for the altered auxin responses in the *hipp* mutants. Using yeast two-hybrid and BiFC analyses we mapped the protein domains involved in the HIPP-ARF interactions and determined that these interactions occur in the cytoplasmic condensates. Finally, we show that HIPP proteins control the stability and therefore transcriptional activity of ARF proteins. We will discuss our findings and recent advances to decipher the mode of HIPP activity in regulating auxin responses.

O-29

Decoding the transcriptional antagonism of AUXIN RESPONSE FACTORs

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Our research focuses on the evolutionary history of the nuclear auxin signalling pathway in plants. Auxins play an essential role in regulating various processes throughout plant life cycle by triggering multiple and complex responses. The three-component auxin signalling system is an excellent model of a simple pathway that generates complex responses in plants. However, it is unclear how this system evolved.

The presence of part of the auxin signalling system in the closest land plant relatives, the streptophytan algae, suggests that at least some of the elements were already present in a common ancestor. To understand the evolutionary trajectory that allowed the emergence of the pathway, we are studying the functionality of the closest orthologs of the system elements in different algal species, with special attention to ARFs.

We are reconstructing the most likely ancestral auxin signalling components using predictive tools and protein-protein and -DNA interaction assays. Initial efforts have been done in the bioinformatics behind ARF DNA binding domain prediction, together with the set-up of medium to high-throughput assays to understand DNA-binding on these elements.

In the future, we aim to assemble them into a functional auxin response system reminiscent to the ancestral systems that evolved into the land plant auxin response system.

O-30

Intrinsic disorder by conformational multistability in auxin co-receptors

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All cascades of auxin-induced molecular events start with the binding of auxin to one of its receptors, whose canonical representative is TIR1. Subsequently, the transcription-regulating AUX/IAAs bind on the TIR1-auxin system, completing a TIR1-ubiquitin E3 ligase complex. Whilst the binding selectivity mechanisms have been investigated, little is known yet about the processes determining the association of the Aux/IAAs with the initial complex, except for the importance of a degron sequence in domain II of all AUX/IAAs. To characterize the formation of the co-receptor complex, it is important to establish the structural characteristics of a full-length Aux/IAA protein. The highly conserved C-terminal PB1 domain of Aux/IAA17 is solved, but knowledge of the N-terminal half is fragmentary because its structure is believed to be intrinsically disordered. We used advanced molecular dynamics simulations to produce a picture of the entire Aux/IAA17 protein and to estimate its interactions with TIR1. Our results suggest that Aux/IAA17 displays multiple semi-folded structures coexisting in different amounts, with the two most abundant ones accounting for more than 90% of the population. Extensive simulations allowed us to estimate a weighted map of contacts between Aux/IAA17 and the TIR1/auxin complex. Our estimates for the ensemble of structures are supported by CD assays, and our predictions for the complex contacts are supported by NMR intensity-loss experiments. Our study suggests the existence of a novel class of intrinsically disordered proteins characterized by conformational multistability, to which the Aux/IAAs belong.

Session Opening Lecture

O-31

Knowns and unknowns in auxin signalling

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The plant hormone auxin is a versatile intercellular signal influencing virtually all aspects 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. One of the key roles of auxin is adaptation of plant growth to gravity, where shoots bend up and roots down. This paradox is based on opposite responses of these organs to the phytohormone auxin, which promotes cell expansion in shoots, while inhibiting it in roots via an unclear signalling pathway and yet unknown downstream cellular mechanism

The well-established canonical auxin signalling involving the TIR1/AFB auxin receptors, Aux/IAA repressors and ARF transcription factors acts in nucleus and mediates gene transcription. However, auxin also triggers cellular responses within seconds or minutes, too fast to rely on transcription. Part of the rapid responses is mediated by the non-transcriptional branch of the TIR1/AFB signalling, but others involve a yet completely unknown mechanism.

Here I will present new and surprising insights into the mechanism of auxin signalling including an ultrafast auxin-triggered protein phosphorylation response and previously unsuspected aspects of TIR1/AFB auxin perception and downstream signalling.

O-32

The mechanisms and functions of rapid auxin responses in Arabidopsis roots

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Recently, our understanding of auxin signaling expanded, and apart from the 'classical' TIR1/AFB nuclear auxin pathway, auxin is perceived in the apoplast by the TMK1 module, and in the cytoplasm by the AFB1 receptor. The mechanistic details of the cytoplasmic pathway have not been fully elucidated. Importantly, the biological significance of rapid auxin responses as well as their interactions with the nuclear auxin signaling, are not well understood.

Here we will present our results explaining the mechanism of subcellular localization of the AFB1 receptor and the downstream signaling pathway. Further, we will focus on the interaction of the AFB1-driven cytoplasmic auxin pathway with the nuclear auxin response. Finally, we will discuss the significance of the rapid auxin response in the roots beyond the context of external auxin application – we will describe the role in root surface pH zonation and gravitropism dynamics.

O-33

Live imaging of cyclic nucleotides in Arabidopsis roots and their role in calcium homeostasis and the ROP GTPase cycle

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Auxin-induced root growth inhibition occurs rapidly, ruling out transcriptional regulation. Recent evidence suggests that cyclic nucleotides are produced by the cyclase activities of auxin receptors TIR/AFBs. However, the role of these important second messengers for rapid responses has been largely overlooked in plants, and their dynamics within plant tissues remain unknown.

To address this, we introduced metazoan cyclic nucleotide sensors based on circularly permuted GFP and monitored cAMP and cGMP levels during root gravitropic bending, root hair development, and auxin exposure. Our results demonstrate the importance of cyclic nucleotides for calcium homeostasis and ROP GTPase activity in plant roots.

In conclusion, our study sheds light on the role of cyclic nucleotides in plant signaling and provides novel insights into the mechanisms underlying rapid responses to auxin in Arabidopsis roots and their relationship with the mechanisms maintaining the root hair tip growth.

O-34

Guanylate cyclase activity of TIR1/AFBs auxin receptors in rapid auxin responses

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The phytohormone auxin acts in growth and development mainly by triggering transcriptional reprogramming. Well-characterized TIR1/AFBs auxin receptors mediate transcriptional auxin responses through degrading the transcriptional repressors Aux/IAAs by their E3 ubiquitin ligase activity after auxin perception. Accumulating evidence indicates that TIR1/AFBs also mediate rapid cellular responses like cytosolic Ca^{2+} transients, plasma membrane depolarization, and apoplast alkalinization. These converge on rapid root growth inhibition and are too fast to be explained by the transcriptional mechanism. Recent discovery of an additional adenylate cyclase (AC) activity for TIR1/AFBs provides a promising avenue to the mechanism underlying these elusive rapid non-transcriptional auxin responses. However, it turned out to be an unexpected twist that AC activity is not essential for rapid auxin responses but crucial for the classical, transcriptional responses. We will present here that TIR1/AFBs also have guanylate cyclase (GC) activity. The GC and AC activities are determined by adjacent but independent GC and AC motifs in the TIR1/AFB C-terminus. Our data suggest that in contrast to AC activity, which is crucial for transcriptional auxin responses, GC activity is specifically involved in rapid non-transcriptional auxin responses. Hence, TIR1/AFBs generate two major second messengers (cAMP and cGMP) in parallel, with each mediating a distinct set of transcriptional and non-transcriptional auxin responses. This unprecedented combination of AC and GC activities in a hormone receptor provides a new paradigm for how a single perception mechanism can mediate a multitude of downstream responses.

Development (shoot)

Session Opening Lecture

O-35

ABP1/ABLs and TMKs are co-receptors for extracellular auxin

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Extracellular perception of auxin has been debated for decades. Auxin binding protein 1 (ABP1) physically interacts with transmembrane kinases (TMKs) and was reported to participate in the perception of extracellular auxin, but its role was controversial because *abp1* knockout mutants were reported to lack major developmental phenotypes. We identified two new apoplastic auxin-binding proteins, ABL1 and ABL2, that exhibit auxin-mediated direct interaction with the extracellular domain of TMKs. Furthermore, ABL1 and ABL2 are functionally redundant and overlapping with ABP1, and genetically interact with TMKs. Evidence for ABP1/ABLs and TMKs acting as co-receptors for extracellular auxin will be presented.

O-36

Fruit shape diversification requires local maintenance of meristem identity and depends on a positive feedback mechanism

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In animals and plants, organ shape is primarily determined by cell division during primordium development followed by cell expansion. Rare examples of post-primordial change in morphology (reshaping) exist and offer accessible and tractable systems to study diversification of organ shape. One such example is the heart-shape formation of *Capsella* fruits that emerges from reshaping of the ovate spheroid gynoecium upon fertilisation. Here we use whole-organ live-imaging to show that dynamic changes in cell division and cell size coupled with local maintenance of meristematic identity drives *Capsella* fruit shape formation. At the molecular level, we reveal an auxin-induced mechanism ultimately descending on variation in a single *cis* regulatory element to mediate a morphological alteration. This element resides in the promoter of the *Capsella rubella SHOOTMERISTEMLESS* (*CrSTM*) gene. Molecular analyses revealed that the CrSTM meristem identity factor positively regulates its own expression through binding to this element providing a feed-forward loop at the position and time when protrusions emerge to form the heart. Presence of the STM binding sequence in *STM* promoters across Brassicaceae species correlates with those undergoing a morphological change of the fruit upon fertilisation. Indeed, our genetic and phenotypic studies showed that the STM binding sequence is required to facilitate the shape transition and reveal a conserved molecular mechanism for organ shape determination.

O-37

Touching PINOID: auxin and calcium-dependent regulation of phyllotaxis at the Arabidopsis inflorescence meristem

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The plant hormone auxin elevates cytosolic calcium levels ([Ca^{2+}]cyt), but how Ca^{2+} signaling promotes auxin-mediated responses is less clear. Here we show that the *Arabidopsis thaliana* plasma membrane (PM)-associated kinase PINOID (PID), a key determinant in polar targeting of PIN FORMED (PIN) auxin efflux carriers, interacts with the calmodulin-like protein CML12/TOUCH3 (TCH3) and 10 closely related calmodulins (CaMs) and CMLs in an auxin-induced, Ca^{2+}-dependent manner. This interaction results in reduced PID kinase activity and its dissociation from the PM to the cytosol. Mapping of the PID-CML/CaM binding and -PM association sites in PID showed that they converge on an amphipathic alpha helix in an insertion in the kinase activation loop. Arginine for alanine substitutions in the amphipathic helix disrupted the PID-CaM/CML interaction and reduced PID PM localization. Based on overexpression phenotypes and complementation experiments, these “untouchable” PID versions were active, despite their reduced PM localisation. However, more detailed observation of the *pid* mutant lines expressing these “untouchable” PID versions detected strong defects in inflorescence phyllotaxis, with two or more flower primordia initiating at the same time. Our results indicate that the calcium-dependent PID-CaM/CML interaction regulates phyllotaxis by integrating auxin and mechanical stress signalling at the *Arabidopsis thaliana* inflorescence meristem.

O-38

bHLH heterodimer complex variations regulate cell proliferation activity in of *Arabidopsis thaliana* meristems

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Root, shoot, and lateral meristems are the main regions of cell proliferation in plants. It has been proposed that meristems might have evolved dedicated transcriptional networks for cell proliferation homeostasis. Here, we show that basic helix-loop-helix (bHLH) transcription factor heterodimers formed between members of the TARGET OF MONOPTEROS5 (TMO5) and the LONESOME HIGHWAY (LHW) subclades are general regulators of cell proliferation in all meristems. Yet, genetics and expression analyses suggest specific functions of these transcription factors in distinct meristems, possibly due to their expression domains determining heterodimer complex variations within meristems, and to a certain extent, to some of them being absent in a given meristem. Target gene specificity analysis for heterodimer complexes focusing on *LONELY GUY* gene targets further suggests differences in transcriptional responses through heterodimer diversification that could allow a common bHLH heterodimer complex module to contribute to cell proliferation control in multiple meristems.

Session Opening Lecture

O-39

Towards mechano-biochemical cellular models of auxin-driven plant growth and development

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Recent advancements in computing power have led to the development of spatio-temporal computer models that simulate pattern formation in plant development. One growth regulator in particular, Auxin, has attracted significant attention from theoreticians and modelers due to its involvement in nearly every aspect of plant life. However, current modeling approaches face notable limitations that hinder our understanding of auxin dynamics during plant organogenesis and development. These limitations include oversimplified tissue geometries, a lack of realistic representation of cell growth, and an often inability to address tissue biomechanics. In this presentation, I will introduce a new approach that addresses these bottlenecks by integrating advanced computer graphics techniques, specifically Position-based Dynamics (PBS), to model the biomechanics of growing plant tissues. By combining biochemical and biomechanical aspects of plant growth and auxin dynamics at the resolution of single cells, this framework offers a comprehensive understanding of plant development. I will demonstrate how this approach can be applied to model various aspects of plant development, including the anisotropic growth of the primary root meristem, graded cell proliferation, leaf and flower initiation, and fruit morphogenesis. This new strategy underscores the importance of integrating tissue mechanics and auxin dynamics in unified computer models to uncover the mechanisms behind anisotropic cell growth and the diversity of organ shapes.

O-40

Fade out: Cytokinin control of reproductive shoot architecture

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Plants must carefully control the number and duration of their reproductive effort in order to produce an optimal number of seeds relative to environmental resources. However, achieving the correct balance of resource allocation between structures requires plants to know how many flowers and fruit they have already formed, in order to determine how many more flowers they can still produce. How does this integration of external signals and internal feedback occur, in order to produce optimal reproductive architecture? Here, we demonstrate the importance of cytokinin distribution within the plant as a potential self-organising system to achieve this balance. We show that cytokinin is vital to maintaining active flowering, both at the level of inflorescence meristem activity, and in the opening of individual flowers. We show that cytokinin modulates every aspect of reproductive development in Arabidopsis, thus tying all reproductive decisions in a single network. We therefore propose a “cytokinin dilution” model, in which the distribution of the of root-derived cytokinin within the shoot connects resource availability to the duration and extent of flowering. We discuss the generalizability of this model, and how it might apply to crop species such as wheat and barley. Our work has important implications for understanding how additional yield can be produced from crops without additional inputs.

O-41

Deciphering the global proliferative arrest: an elusive link between plant reproduction and longevity

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Proliferation of meristematic cells defines the final shape of body as well as plant longevity. In many species, including annual crops and model *Arabidopsis thaliana*, inflorescence meristem (IM) activity and plant longevity are coupled with reproduction. Once plant produces a predetermined number of seeds, activity of all IMs is inhibited, and new flowers cease being formed. This phenomenon is termed global proliferative arrest (GPA) and indicates existence of a systemic signalling mechanism that measures number of produced seeds and communicates it to meristems.

GPA has important implications for crop yield, but little is known about its molecular underpinning. We attempt to dissect this process by quantitative imaging of inflorescence meristem at different stages of plant development using a unique technology that enables 3D reconstitution of meristems from light sheet microscopy data. We focus on changes in hormonal signalling and proliferative activity in IM during its progression towards GPA and propose a model for meristem arrest.

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O-42

Competing differentiation gradients coordinate fruit morphogenesis

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Morphogenesis requires the coordination of cellular behaviors along the developmental axes. In plants, gradients of growth and differentiation are typically established along a single longitudinal primordium axis to control organ shaping. Here we combine quantitative live-imaging at cellular resolution with genetics, chemical treatments, and modeling to understand the formation of *Arabidopsis thaliana* female reproductive organ (gynoecium). We show that, contrary to other aerial organs, gynoecium shape is determined by two competing differentiation gradients positioned along two orthogonal axes. An early mediolateral gradient, dependent on meristematic activity in the medial domain, controls the valve morphogenesis while simultaneously restricting an auxin-dependent, longitudinal gradient to the style. This gradient competition serves to fine-tune the common developmental program governing organ morphogenesis to ensure the specialized function of the gynoecium.

O-43

Phyllotactic transitions in strawberry flowers – from spirals to whorls to spirals

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Angiosperm flowers show enormous diversity in terms of number and arrangement of their floral organs. In most flowers, floral organs are arranged in a whorled phyllotaxis, with each organ types forming in concentric whorls. By contrast, in some basal angiosperm species, floral organs are organized in continuous left- and right-turning spirals, irrespective of their identities. So far, little is known about the patterning mechanism differentiating whorled versus spiral phyllotaxis. Here, we elaborate woodland strawberry (*Fragaria vesca*) as a model species since its early flower development undergoes multiple transitions: first from spiral to whorled, followed by whorled to spiral in phyllotactic patterns. Combining high-resolution synchrotron-based micro-CT and confocal live imaging of transgenic reporter lines, we show the dynamic growth of the strawberry flower meristem in 3D. We found that the transitions between spiral to whorled, or whorled to spiral arrangement of floral organs are marked by changes in auxin patterns during the initiation of organ primordia. Such differences are mediated by polar auxin transport and correlate with expansion rate of the meristem governed by cytokinin. Finally, we aim to integrate the developmental data with computational modelling to explain phyllotactic transitions in strawberry flowers.

Development (vasculature)

Session Opening Lecture

O-44

Single-cell transcriptome analysis of protophloem reveals dynamic pattern of hormonal regulation

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Vascular tissue development is guided by hormonal signalling, most recognizably by auxin and cytokinin. During early embryogenesis, onset of cytokinin response promotes separation of pre-procambial tissue into xylem and phloem/procambium domains. Developing phloem cells reside within the cytokinin domain and expression of essential phloem fate regulators, like PEAR transcription factors, is dependent on cytokinin. Detailed transcriptome analysis of the phloem cell lineage revealed how root meristem-wide signals are translated into important decisions on the tissue type level, e.g. onset of APL expression and subsequent transition to differentiation dictated by diminishing concentration of PLT proteins. To understand how auxin and cytokinin signalling may contribute to phloem development we analysed expression pattern of auxin and cytokinin signalling pathway components along the phloem developmental trajectory. Expression of cytokinin receptors as well as A type ARFs was mostly confined to the early phloem suggesting a pattern of dynamic changes in hormonal response. We experimentally determined the link between cell-autonomous auxin signalling and the expression of APL-pathway genes which mark developmental transition to phloem differentiation.

O-45

Developmental trajectories and auxin efflux control

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Polar auxin transport in the Arabidopsis root tip maintains high auxin levels around the stem cell niche that gradually decrease in dividing stem cell daughters but increase again once they transition towards differentiation. Protophloem sieve elements differentiate earlier than other proximal tissues and employ a unique 'canalization' mechanism to accumulate auxin. The associated molecular machinery is thought to balance auxin efflux with auxin retention, which is necessary for properly integrated and correctly timed sieve element differentiation. However, the machinery's net output at the cellular level remains unclear. Recently, we found that expression of its three components in the ectopic context of developing xylem vessels, in distinct combinations, affects root growth vigour by changing the dynamic turnover of auxin efflux carriers. Our data provide further insight into the dosage-sensitive regulatory interactions between the components and show that their dominant collective output is the localized reduction in auxin efflux carrier abundance, by stimulating their clathrin-mediated endocytosis. Moreover, we found that ectopic assembly of the machinery accelerates the trajectory of xylem vessel development. Our data thus provide direct evidence that local manipulation of auxin efflux alters the timing of cellular differentiation in the root.

O-46

Hormonal crosstalk during cambium development

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Vascular cambium provides radial growth in plants. This lateral meristem produces secondary xylem inwards and secondary phloem outwards. Our earlier studies revealed that both xylem and phloem originate from a single bifacial stem cell. Next to stem cells, xylem identity is defined by an auxin maximum, and consequential expression of HD-ZIP III transcription factors. Clonal activation of high auxin signalling in phloem parenchyma led to differentiation of the clone as xylem vessel. Cells adjacent to clone obtained cambium stem cell identity. Based on these studies, we proposed that cells with auxin maximum define an organizer, which positions stem cells in the adjacent cells. During my presentation, I will explain how a few other plant hormones connect to the regulatory network defining the stem cell organizer. Previous studies have shown that Gibberellic acid (GA) promote secondary xylem production in vascular cambium. By combining molecular genetics and confocal imaging, we discovered that GA promotes polar auxin transport along the root. Subsequently, auxin signalling levels increase and broaden in cambium. This broadened auxin maximum forces larger number of cells in cambium to differentiate as xylem, thus explaining the enhanced xylem production after GA treatment. Additionally, I will explain how peptide gradient originating from the phloem converge with the auxin signalling to position the stem cells. Together, our studies reveal how various hormonal pathways converge in cambium to position the stem cells and regulate their differentiation into xylem and phloem.

O-47

PDK1-activated AGC1 kinase autocatalytic circuits pattern the vascular network in *Arabidopsis thaliana*

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Due to the high degree of plasticity, plant vascular networks have been fascinating biologists for centuries. Vascular networks disseminate in the developing leaf primordia through the successive incorporation of undifferentiated ground cells into an interconnected and polarized procambial cell strand. Positional information on procambial cell fate commitment within the developing primordium underlies the spatiotemporal distribution of the plant hormone auxin. Multiple lines of evidence propose an elaborate interaction of auxin biosynthesis, auxin cell-to-cell transport and local auxin transcriptional interpretation in the developing tissue culminating to give rise to the functional vascular network morphology. However, the molecular framework underlying such complex coordination of events is still far from understood.

Here, we explored the role of AtAGC1 kinases activated downstream of AGC kinases PHOSPHOINOSITIDE-DEPENDENT KINASE 1 AtPDK1 during vascular network morphogenesis. We describe the spatiotemporal activity of the AtAGC kinase cascade during leaf vascular patterning, its dynamic regulation upon auxin transport and auxin signaling perturbation and directly link its catalytic activity to procambial cell fate determination and morphogenesis. Finally, we propose that AtAGC kinases function within an autocatalytic kinase circuit that operates to interpret local signaling cues within the homogeneous subepidermal ground tissue of the developing leaf primordia. This serves to pattern procambial cell fate commitment and to elicit coordinated procambial cell morphogenesis. With the work presented, we uncover the previously unknown function of AtAGC1 kinases during vascular tissue morphogenesis, the functional characterization of which will help to decipher the molecular nature of vascular network morphogenesis and its captivating plasticity.

Development (root)

O-48

Interspecific root patterning diversity: The cytokinin route

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Asymmetric cell division (ACD) is a most successful strategy in multicellular organisms to generate cell diversity. How differences in the control and positioning of ACD generate patterning diversity between species is however still unclear. The ground tissue (GT), formed by endodermis and cortex(es), represents an optimal model to clarify the role of ACD in controlling root patterning, as cortical layer number varies among species. Comparing two closely-related plants, *Arabidopsis thaliana* (1 cortex layer) and Cardamine hirsuta (2 cortex layers), we showed that the species-specific spatial distribution of microRNAs 165 (miR165), 166 (miR166) and of their targets HOMEODOMAIN LEUCINE ZIP PROTEIN III (HD-ZIPIII) transcription factors controls the ACDs that determines the number of cortical layers. However, the mechanisms through which miR165/166/HD-ZIPIII module controls ACD remain vague. Here we show that interspecific differences in spatial distribution of miR165/166/HD-ZIPIII module promote and position ACDs via generation of species specific cytokinin activity domains. We demonstrate that the synergistic and coordinated activity of HD-ZIPIII with another pathway controlling GT ACDs, the SHORTROOT/SCARECROW pathway, is required for the formation of proper ACD in the GT. All together our data underlie how differences in ACD position and promotion generate patterning diversity in organs.

O-49

The role of the plant cuticle during apical hook development

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In order to protect the apical meristem from mechanical damage while emerging from the soil, dicotyledonous plants develop a transient structure at the upper end of the hypocotyl named apical hook. Apical hook development involves differential growth in a well-defined time frame, including formation, maintenance and opening phases. Among the cellular signaling mechanisms involved in the regulation of apical hook development, those involving phyto-hormones such as auxin, ethylene and gibberellins are certainly the best characterized. However, the structural modifications implicated in this process are not clearly understood yet. In a search for new players in the modulation of hook development, we found that three cuticle-related mutants, *defective in cuticular ridges* (*dcr*), *cytochrome p450, family 77, subfamily a, polypeptide 4* (*cyp77a4*) and *bodyguard* (*bdg*) display defective apical hook development and wavy hypocotyls. TEM analysis revealed a thinner cuticle in the mutants compared to that of the wild type at both sides of the apical hook. Interestingly, these mutants are characterized by increased cell division events, reduced cell elongation and reduced auxin levels during apical hook development. Auxin treatment further hastens apical hook opening while partially mitigates the hypocotyl waviness. Our data suggest that cuticle is important for proper apical hook development, however, the mechanisms underlying its role remain unclear, as well as its relationship with auxin. To investigate these mechanisms, we have generated lines with spatially localized cuticle degradation that will be used to pave the way to a better understanding of the role of the cuticle in apical hook development.

O-50

Characterization of early adventitious rooting via HYSPARIN, a shoot-specific inducer of auxin responses

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Lateral root (LR) branching and adventitious root (AR) formation are agronomic important traits determining crop yield and the efficiency of clonal propagation. While the initiation of LR has been studied intensively, the ontogeny of AR and its relationship to the initiation of LRs remain largely unknown. We identified from a collection of small molecule named Hypocotyl Specific Adventitious Root INducer (HYSPARIN; HYS) based on a strong AR inducing capacity without pronounced effects on primary root growth and lateral root branching. HYSPARIN activates the pericycle in Arabidopsis hypocotyls, yet is not a typical auxin as it does not trigger a typical rapid molecular response of DR5-reporter activation, DII-Venus degradation or Ca^{2+} signalling. I will present our findings of how this chemical has allowed to characterize the early events during adventitious root induction, and to study how light represses adventitious rooting

O-51

Does auxin modulate xyloglucan endotransglucosylase activity directly in the cell wall?

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Auxin is a key plant regulatory molecule acting upon many cellular processes. How exactly auxin regulates cellular elongation and underlying cell wall dynamics is still not fully understood. Previously, by using click chemistry-based tracking and azido-IPA, we identified putative auxin-binding sites in the outer cell walls of expanding root cells. This observation prompted us to investigate the intriguing possibility of some extracellular auxin activity in those cell wall microdomains. Based on data from the literature, apoplastic xyloglucan endotransglucosylase/hydrolases (XTHs) —exhibiting xyloglucan:xyloglucan endotransglucosylase (XET) activity—became our prime suspect of a direct auxin target. We studied the auxin effect on XET activity using both *in vitro* and *in vivo* methods. Protein extracts and frozen tissue sections served as biological systems with disrupted intracellular signalling. Adding IAA and NAA to these samples increased XET activity by app. 20%, whereas 2,4-D and IBA showed no effect, even at high concentrations. Fluorescent xyloglucan oligosaccharides (F-XGO)—a substrate for XTHs—are incorporated precisely at the site where root cell elongation is initiated, co-localizing with auxin binding sites in the outer epidermal cell wall domain. Altered incorporation of F-XGO has been observed after (i) exogenous application of IAA, (ii) a potent XTHs inhibitor, and (iii) in the loss and gain-of-function mutants of PIS1/PDR9/ABCG37 auxin transporter. Our data support a hypothesis that the epidermal outer lateral cell wall domain is a hot spot for auxin-mediated cell wall remodeling with a function in establishing proper mural adjustment required for cell morphogenesis but independent of acid growth.

O-52

The SCFKMD family of F-box proteins promote maintenance of the root quiescent center downstream of cytokinin signaling

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Cytokinin plays a crucial role in plant growth and development. Post-embryonic root growth is tightly controlled by the cytokinin-auxin interaction in the root apical meristem (RAM). The meristem cells and stem cells in the root apical meristem are derived from the quiescent center (QC)—a group of slowly dividing, undifferentiated cells. Here we demonstrate that the KISS ME DEADLY (KMD) family of F-box proteins—a component of the E3 ubiquitin ligase complex and a negative regulator of cytokinin signaling—suppress QC division and promote QC maintenance. The *kmd* mutant seedlings show increased QC division and aberrant divisions that result in a reduced RAM size. In contrast, seedlings overexpressing members of the KMD family exhibit insensitivity to cytokinin-induced QC division compared to the wild type. GUS fusions to KMD family promoters clarify regions of the root in which the genes are expressed. KMD1 and KMD2 proteins fused to GFP localize to QC and root meristem zone respectively. These data suggest *KMD* genes act downstream of cytokinin to control QC division, maintenance, and meristem size.

Development (evolution)

O-53

A B4-RAF-like kinase mediates a deeply conserved, rapid auxin response

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The plant signaling molecule auxin triggers both fast and slow cellular responses across the plant kingdom, including both land plants and algae. A nuclear response pathway mediates auxin-dependent gene expression, and controls a range of growth and developmental processes in land plants. It is unknown what mechanisms underlie both the physiological responses occurring within seconds, and the responses in algae, that lack the nuclear auxin response pathway. We discovered a fast proteome-wide phosphorylation response to auxin across 5 land plant and algal species, converging on a core group of shared target proteins. We find conserved rapid physiological responses to auxin in the same species and identified a RAF-like protein kinase as a central mediator of auxin-triggered phosphorylation across species. Genetic analysis allowed to connect this kinase to both auxin-triggered protein phosphorylation and a rapid cellular response, thus identifying an ancient mechanism for fast auxin responses in the green lineage.

O-54

Evolution of a non-canonical auxin signalling pathway controlling lateral organ development

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Classical studies have established a 'canonical' auxin signalling pathway involving the degradation of repressors facilitated by auxin, but recent studies have also uncovered multiple non-canonical signalling pathways. One such pathway, the ETTIN (ETT)-mediated pathway, involves an atypical auxin response factor (ARF) that is able to sense auxin directly through its specialised middle region ('ES' domain) to modulate target gene regulation and protein-protein interactions. ETT and its close paralogue, ARF4, function to different degrees of redundancy in leaf and gynoecium development. Nonetheless, the origin of ETT-mediated auxin signalling, and its contribution to leaf and flower development remains unclear. Therefore, this project aims to elucidate the evolution and mechanism of the ETT-mediated pathway. Our phylogenetic analyses reveal that the ETT/ARF4-like clade diverged from the rest of the B class ARFs in the last common euphyllophyte ancestor, and subsequently separated into individual ETT and ARF4 clades in the last common angiosperm ancestor. Protein-protein interaction assays of ETT/ARF4(-like) orthologues suggest that the auxin-sensing ability of ETT is a neofunctionalisation exclusive to the angiosperms. Complementation lines of *ett-3* and *ett-3 arf4* loss-of-function mutants elucidate the origin of ETT/ARF4's role in vegetative and reproductive development. Finally, an RNAseq of species from key phylogenetic lineages treated with auxin and/or a canonical pathway inhibitor will uncover the conservation of the ETT-mediated pathway in the land plants.

O-55

Evolution of plant architecture through changes in auxin movement control

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The successful colonization of land by plants was accompanied by the diversification of their branching architecture. The phytohormone auxin is a major regulator of branch initiation and has a similar inhibitory role in flowering plants and mosses, two major land plant lineages that diverged from their most recent common ancestor several hundred million years ago. PIN-mediated polar auxin transport is crucial for auxin function in flowering plant branching control. Long-range tropic auxin gradients are sustained locally through the regulation of cell-to-cell connectivity at the level of plasmodesmata, although this is comparatively a minor pathway. In the moss *Physcomitrium patens*, an extant representative of early land plants, PIN proteins have a minor role in leafy shoot branching control and the symplasmic pathway could instead represent the main route for auxin movement in the stem. Using a combination of developmental genetics and computational modeling, we explore the role of symplasmic fields and plasmodesmal gating in auxin movement, and thereby assess their contribution to the evolution of plant morphogenesis.

O-56

How auxin regulates cellular growth dynamics to shape a leaf-like organ

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Leaves are vascular plant organs, optimized for photosynthesis. Auxin plays a critical role during leaf development, controlling its patterning as well as tuning cellular growth and differentiation. Auxin is also important for the development of phyllids, the bryophyte leaf-like organs. However, its precise role in this process remains elusive. Here we use a combination of live imaging, genetics, chemical treatments, and modeling to understand how auxin controls phyllid development in the model moss species, *Physcomitrium patens*. We tracked basal and upper phyllid morphogenesis from a single initial cell until full maturity to uncover the cellular growth dynamics underlying this process. We explored the role of auxin by recording auxin synthesis and transport patterns throughout phyllid development and integrated our experimental data into a computational model of a growing phyllid. Our results elucidate the cellular basis of auxin function in the regulation of developmental decisions allowing moss leaf-like organs to reach their final shape and structure.

Interactions and Cross-talk

Session Opening Lecture

O-57

On the Integration of External Cues into Auxin-Dependent Growth Programs

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The phytohormone auxin plays a critical role in the growth and development of plants. However, the precise subcellular mechanisms governing the control of auxin remain largely elusive. We investigate the subcellular processes that enable the integration of multiple external cues into auxin-dependent growth programs. Specifically, we focus on the role of PIN-LIKES (PILS) proteins as facilitators of auxin transport at the endoplasmic reticulum (ER), which ultimately govern nuclear auxin signaling rates and growth rates. Our work highlights the coordinative role of PILS proteins in integrating environmental signals into nuclear auxin signaling output.

O-58

PhyA-dependent phosphorylation of AHP3 regulates cytokinin-mediated photomorphogenesis

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Plants, being sessile organisms, have evolved complex molecular responses to adapt to ever-changing environments while balancing their growth with survival. Previously, we demonstrated that photoreceptor molecules called phytochromes (phy) are involved in crosstalk with components of the multistep phosphorelay (MSP) pathway, which is a crucial signaling nexus for plant growth and stress responses. However, the mechanism of this crosstalk remains elusive. Here, we present evidence of the negative impact of phyA on the MSP, resulting in a transient reduction in cytokinin signaling activity. We identified phyA as interacting with and phosphorylating ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 3 (AHP3) exclusively among all AHPs. We detected physical interaction between the PAS-A domain of phyA and AHP3, uncovering the interaction specificity between light and cytokinin signaling components. We established that phyA phosphorylates conserved serine residues of AHP3, attenuating the expression of type-A response regulators and further modulating cytokinin-induced hypocotyl elongation under far-red light conditions. Additionally, overexpression of AHP3 displayed notable phenotypic characteristics under drought stress. Nonetheless, the unique phenotype of AHP3 overexpressing plants relied on functional light signaling mediated via phyA. Thus, phyA-dependent regulation of AHP3 provides a unique mechanism for plant developmental reprogramming under the changing environment.

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O-59

mRNA N6-adenosine methylation (m6A) integrates multilevel auxin response and ground tissue development

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N6-methyl adenosine (m^6A) is the most common mRNA modification that plays a critical role in regulating transcript stability and overall gene expression. Our study investigates the connection between m^6A and auxin pathways in *Arabidopsis thaliana*. We characterize the auxin-related phenotypes and auxin resistance of the hypomorphic mutants with the impaired status of m^6A. Next, through an extensive analysis of the published transcriptome-wide m^6A datasets, we demonstrate that m^6A is present in many auxin-related transcripts. Quite exceptional is the group of SAUR transcripts that, in contrast to other categories, lack methylation marks completely. We further show that transcripts encoding the key components of the auxin signaling pathway, including the TIR1/AFB receptors and ARF transcriptional regulators, might be particularly affected by the m^6A-mediated regulation. We also observe the correlation between the methylation status of IAA amidohydrolase transcripts and the moderately altered internal levels of IAA metabolites. In addition, we reveal that the lack of m^6A leads to defects in the endodermal patterning of the primary root due to the impaired timing of periclinal cell divisions. Notably, these defects can be reverted by inhibition of auxin signaling. Overall, our data highlight the crucial role of m^6A in regulating multiple aspects of auxin-dependent processes.

O-60

Glutathione enhances auxin sensitivity in Arabidopsis roots

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Root development is regulated by the tripeptide glutathione (GSH), a strong non-enzymatic antioxidant found in plants but with a poorly understood function in roots. Here, Arabidopsis mutants deficient in GSH biosynthesis (*cad2*, *rax1,* and *rml1*) and plants treated with the GSH biosynthesis inhibitor buthionine sulfoximine (BSO) showed root growth inhibition, significant alterations in the root apical meristem (RAM) structure (length and cell division), and defects in lateral root formation. Investigation of the molecular mechanisms of GSH action showed that GSH deficiency modulated total ubiquitination of proteins and inhibited the auxin-related, ubiquitination-dependent degradation of Aux/IAA proteins and the transcriptional activation of early auxin-responsive genes. However, the DR5 auxin transcriptional response differed in root apical meristem (RAM) and pericycle cells. The RAM DR5 signal was increased due to the up-regulation of the auxin biosynthesis TAA1 protein and down-regulation of PIN4 and PIN2, which can act as auxin sinks in the root tip. The transcription auxin response (the DR5 signal and expression of auxin responsive genes) in isolated roots, induced by a low (0.1 µM) auxin concentration, was blocked following GSH depletion of the roots by BSO treatment. A higher auxin concentration (0.5 µM) offset this GSH deficiency effect on DR5 expression, indicating that GSH deficiency does not completely block the transcriptional auxin response, but decreases its sensitivity. The ROS regulation of GSH, the active GSH role in cell proliferation, and GSH cross-talk with auxin assume a potential role for GSH in the modulation of root architecture under stress conditions.

Session Opening Lecture

O-61

Auxin-cytokinin nexus: the shady side of the story

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Light is essential for photosynthesis and morphogenesis, two processes that are essential for plant growth. Competition from nearby plants reduces the amount of photosynthetically active radiation, which causes morphological changes in plants such as hypocotyl elongation in seedlings and petiole elongation in adult plants to capture more light than the competing neighbours. Shade Avoidance Responses (SARs) are the collective name for these adaptive responses. SAR is crucial for plants to survive in a stochastic environmental condition. However, activation of SARs is an important developmental decision as activation of SARs risk seed production and defence against pathogens and herbivores. Therefore, signalling mechanisms that connect internal sugar reserve and SAR-regulating signalling machinery will help plants in reaching a resource-based decision on SARs during competition. We discovered that photosynthetically produced sugars control the elongation of the hypocotyl in a dose dependent manner. We also discovered that auxin and cytokinin signalling played a crucial part in this phenomenon. Auxin signalling in the shade was encouraged when the sugar content was low, resulting in hypocotyl elongation. Less auxin signalling and more cytokinin signalling occurred at higher sugar concentrations leading to inhibition of hypocotyl elongation. We discovered that this sugar-mediated control of phytohormone signalling integrates at the PHYB-PIF4 axis by controlling PHYB nuclear localization and PIF4 binding to target promoters. Our research reveals an important regulatory relationship that links carbon fixation and photosynthesis to the responses that plants have to competition.

O-62

"CYTOLENE" – a new modulator of plant growth and development?

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The interconnected action of cytokinins and ethylene in the control of plant growth was demonstrated. However, the extent of the crosstalk and the underlying molecular mechanisms remained mostly elusive. We and others' have demonstrated that multistep phosphorelay (MSP) pathway, previously thought mainly to mediate cytokinin signaling, is under control of ethylene through the histidine kinase (HK) activity of ETHYLENE RESPONSE 1 (ETR1). Here we show that although ETR1 is an active HK, its receiver domain ($ETR1_{RD}$) is structurally and functionally unable to accept the phosphate from the phosphorylated His in the ETR1 HK domain ($ETR1_{HK}$) to initiate the phosphorelay to ARABIDOPSIS HISTIDINE-CONTAINING PHOSPHOTRANSMITTERs (AHPs), the next downstream link in MSP signaling. Instead, ETR1 interacts with another HK ARABIDOPSIS HISTIDINE KINASE 5 (AHK5) and transfers the phosphate from $ETR1_{HK}$ through the receiver domain of AHK5 ($AHK5_{RD}$), and subsequently to AHP1, AHP2 and AHP3, independently of the HK activity of AHK5. We show that AHK5 is necessary for ethylene-initiated, but not cytokinin-initiated, MSP signaling *in planta* and is involved in the hormonal control of root growth. Furthermore, we have identified novel mechanism of transcriptional regulation based on the interaction of members of ethylene and MSP signaling pathways in the spatial-specific control of cytokinin-induced ethylene biosynthesis, mediating the cytokinin-induced, ethylene-regulated root growth. We propose that the aforementioned regulatory network represents a molecular basis for the existence of previously proposed morphogenic field combining the properties of both cytokinins and ethylene, controlling, together with auxin, root growth and patterning.

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O-63

Cytokinin-production by the rhizosphere bacterium *Pseudomonas fluorescens* G20-18 contributes to interkingdom priming of tomato for enhanced drought stress responses

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Plant growth-promoting rhizobacteria improve growth and tolerance to pathogens but their role in abiotic stress resilience remains underexplored. Thus, the interaction of the cytokinin-producing *Pseudomonas fluorescens* G20-18 was assessed with tomato. Root inoculation stimulated shoot and root growth. G20-18 inoculated plants also showed improved drought stress responses with higher leaf chlorophyll, abscisic acid content and stomatal closure than controls. Deep cell physiological phenotyping via activity profiling of key enzymes of carbohydrate and antioxidant metabolism revealed that the beneficial impacts are reflected in distinct bio-signatures. The increase of antioxidant enzyme activities and total antioxidant capacity correlated with elevated levels of relevant secondary metabolites. RNA sequencing revealed distinct qualitative and quantitative differences in gene regulation. The number of genes differentially regulated in response to G20-18 was approximately sevenfold higher during drought stress, indicating that G20-18 primed the plants for a much stronger systemic drought stress response. The regulated genes are related to phenylalanine metabolism and other key processes linked to plant growth, development and drought stress resilience. A role of the ability of G20-18 to produce the phytohormone cytokinin for the interaction was established by the cytokinin-deficient biosynthesis mutants CNT1 and CNT2. In comparison with G20-18, the inoculation with CNT1 resulted in a reduced number of differentially regulated genes. The relevance of the microbial cytokinin production was also evident from differences in growth and specific cell and ecophysiological parameters. These findings demonstrate the ability of G20-18 to prime for improved drought stress responses and a role of interkingdom signalling by bacterial-derived cytokinins.

O-64

Cytokinin auxin interactions during gametogenesis and embryogenesis in tomato: Do they always act antagonistic?

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Hormones play a pivotal role in most physiological processes in plants. This research aimed to elucidate the role of auxin and cytokinin (CK) and their interaction during pollen and seed development. We used transcriptome (RNA-Seq) and metabolome (UPLC-ESI-MS/MS) analysis to explore hormonal regulation during gametogenesis and embryogenesis in tomatoes. To better understand the interaction between auxin and CK, we follow the auxin response factor (*PIAA::Scarlet-N7*) and CK response factor (*TCSv2::Neon-N7*) expressed in the same plants during flower and fruit development. Our research indicates that tomato gametogenesis and embryogenesis are auxin and CK-regulated processes. Interestingly we found novel CK response factor expression patterns in tapetum, around the embryo sac, and in the pollen cells. Suggesting CK plays a vital role during male and female gametogenesis. Our study further demonstrates that auxin and CK interact and do not always act antagonistically, and their interaction patterns vary between flower and fruit cells and organs during their development.

Responses to Environmental Stimuli

Session Opening Lecture

O-65

Manipulation of cytokinin-salicylic crosstalk for increased plant growth and pathogen resistance

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Phytohormones are essential regulators of development and response to biotic and abiotic stresses. Activation of the plant immune system by pathogen attack often results in changes in plant growth, frequently leading to smaller plants with reduced seed set, a phenomenon known as the growth-defense trade-off. Cytokinin, a hormone known for its role in the regulation of cell division and plant growth, also has an important role in the activation of defense against pathogens through a synergistic interaction with the defense hormone salicylic acid. We show that the crosstalk between these two phytohormones also regulates plant development. Our data suggests that modifying the cytokinin-salicylic acid crosstalk can increase reproductive growth and pathogen resistance. Further experimentation and investigation into the mechanistic interactions mediating the balance between plant growth and defense could lead to implementation of phytohormone crosstalk engineering to target specific traits in crop species.

O-66

The GH3 protein from the clubroot pathogen *Plasmodiophora brassicae* causes hormone-related phenotypes in *Arabidopsis thaliana*

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The control of hormone homeostasis is important for all growth and developmental processes. During plant – pathogen interactions auxin can either act as a signal in defense responses or contributes to tumorous disease symptoms. The clubroot disease is caused by a biotrophic protist that transforms the host roots into a tissue with strong hypertrophy and hyperplasia symptoms depending on auxins, cytokinins and brassinosteroids. *Plasmodiophora brassicae* is difficult to study due to its obligate biotrophic nature. Several plant hormone-metabolizing genes were found in the genome of *P. brassicae*. One of these has been assigned to the group of *GH*3 genes encoding proteins of auxin amino acid conjugate synthetases. The PbGH3 protein can convert indole-3-acetic acid (IAA) and jasmonic acid (JA) to a variety of amino acid conjugates, but not salicylic acid and the enzyme is very promiscuous towards the amino acid co-substrates. The *GH3* gene has high conservation across all worldwide *P. brassicae* isolates, including in the promoter region. Auxin and Me-JA responsive elements were found in the short promoter sequence. The host *Arabidopsis thaliana* was transformed with a construct constitutively expressing *PbGH3* and the resulting transgenic population analyzed for its phenotype and hormone profile. Lines with confirmed protein production had altered seedling root growth and adult plant inflorescence architecture. The infection severity of the transformants was not significantly altered. Hormone profiles for IAA, JA and respective conjugates resulted in complex patters, but overall transgenics had lower free IAA and JA and higher IAA-Glutamate, but not IAA-Aspartate levels. JA-Isoleucine decreased.

O-67

How hormone homeostasis contributes to stress resistance in plants

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The plant hormone auxin plays a key role in almost all aspects of plant growth and development, including environmental adaptive responses. Cellular auxin levels are tightly regulated by the interplay between homeostasis, signalling and transport. This creates a cell to cell gradient of auxin, which dependent on tissue and developmental context is translated into developmental outputs. A fundamental way cellular auxin levels are regulated is by auxin degradation. Primarily conjugation to glucose or amino acids, the latter being oxidised by two enzymes in *Arabidopsis thaliana* named DIOXYGENASE FOR AUXIN OXIDATION 1/2 (AtDAO1/2). This work aims to understand how AtDAO1/2, and ultimately auxin degradation, contributes to adapting root architecture to environmental stress. Auxin helps optimise root architecture to maximise resource acquisition while limiting the impact of increasingly common abiotic stresses. Our study induced low water and high salinity environments on *Atdao1/2* knock out and overexpression lines to establish differences in root growth, development, and gene expression. The *dao1-1* knock out line displayed altered root architecture, which was amplified by the presence abiotic stress. This was reflected in cell changes in the root meristematic tissue. Understanding how auxin gradients form to regulate cell differentiation, will provide important insight into basic plant development and identify targets for crop breeding programmes.

O-68

Cytokinin in response to environmental cues

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Cytokinin is a multifaceted plant hormone that plays an important role in diverse plant growth and development processes and participates in mediating interactions with the environment. Our results indicated a prominent role for cytokinin in the regulation of thermomorphogenesis, cold stress, ROS metabolism, nutrition uptake, drought stress, biotic stress, and light signaling. We found the impact of cytokinin on hydrogen peroxide content, amino acid metabolism, and the phenylpropanoid pathway, and our cross-species comparison confirmed that these effects are evolutionarily conserved. In addition to the well-described mechanisms based on gene regulation, our proteome analysis identified several candidates that could be considered master regulators of the cytokinin response, including ribosomal proteins and members of the HSP70 protein family.

O-69

Antigravitropic PIN polarization maintains non-vertical growth in lateral roots

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The growth angle of lateral root and shoot branches is a fundamental determinant of plant form and the capacity of root and shoot systems to capture the resources required for growth. In many cases, branch growth angles are set and maintained in reference to gravity, a quantity known as the gravitropic setpoint angle (GSA). Non-vertical GSAs are fascinating because they require that organs can effect tropic growth both with and against the gravity vector in order to maintain stable angled growth. Here, we will describe the work we have done to uncover the molecular mechanisms underpinning GSA control in the lateral root and in particular, the role of phosphoregulation of PIN proteins in the lateral root columella via the phosphatase subunit PP2A/RCN1. We will also discuss how this work has led to novel genetic technologies to optimise root system architecture for sustainable crop production.

O-70

Cytokinin regulates energy utilization in *Botrytis cinerea*

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Cytokinin (CK) mediates plant immunity and disease resistance. Some phytopathogens have been reported to secrete CKs, and may manipulate host CK signaling during pathogenesis. In recent work, we demonstrated that CK directly inhibits the growth, development, and virulence of fungal phytopathogens, by dis-regulating the cell cycle and reducing cytoskeleton organization and cellular trafficking in the fungus. Investigating the effects of CK on the biology of the phytopathogenic fungus *Botrytis cinerea* (*Bc*), we found that CK possesses a dual role in fungal biology. In a nutrient-rich environment, CK strongly inhibited *Bc* growth and de-regulated cytoskeleton organization. This effect diminished as nutrient availability decreased. In its second role, we show that CK can promote glycolysis and energy consumption in *Bc*, both *in vitro* and *in planta*. In contrast with the inhibitory effects of CK on the fungal cell cycle and cellular trafficking, glycolysis and increased oxidation mediated by CK in *Bc* were stronger with waning nutrient availability. The metabolic effects of CK on the fungus likely reflect the role of plant CK during early fungal infection. In addition to the plant producing CK during its interaction with the pathogen for defense priming and pathogen growth inhibition, the pathogen likely exploits this increased CK to boost its metabolism and energy production, in preparation for the necrotrophic phase of the infection.

O-71

A new framework for root gravitropic response kinetics

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A widely conserved trait in plants is the ability to adapt their post-embryonic development to environmental cues, which include the gravity vector. In this context, angle dependence is necessary for the establishment of plant architecture, a fundamental trait for crop improvement. While the first theory on angle dependence was put forward over 200 years, the debate regarding the nature of angle dependence is ongoing. We developed a high-throughput imaging system for the analysis of Arabidopsis primary root reorientation kinetics. Using this new tool, we were able to detect a previously undescribed complexity of the gravitropic response. In spite of this complexity, we show that angle, magnitude of the gravitropic response and auxin asymmetry are highly correlated across the entire response, suggesting that the Cholodny-Went model still applies. By means of computational modelling, we suggest that the response can be described by a new angle-dependent viscoelastic model, with time-dependent behavioural regimes. Combined, our work provides a novel coherent framework for understanding the biophysical and molecular basis of gravitropism.

O-72

Gravitropism and thermomorphogenesis of lateral roots

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The root system provides access to nutrients and water, and its architecture is central to plant productivity. One of its building blocks is the angular growth of lateral roots (LRs). The angle to the gravity vector at which the organ growth direction is maintained is called the gravitropic set-point angle (GSA). Whereas primary roots typically grow towards gravity (GSA = 0°), the LRs partially suppress gravitropic growth (higher GSA values), defining the degree of the root system's radial expansion.

Here we present that the sensing of the ambient temperature regimes determines the gravitropic growth of LRs in *Arabidopsis thaliana*. We previously showed that asymmetric cytokinin signalling acts as an anti-gravitropic signal in LRs, counterbalancing auxin-dependent gravitropism. Here we reveal how cytokinin and auxin signalling contributes to the temperature-sensitive GSA of emerged LRs. To gain a better molecular understanding of the temperature-dependent gravitropic and anti-gravitropic regulation of the LRs, we assessed the GSA deviation in natural accessions of *Arabidopsis thaliana*. We analysed more than 200 *Arabidopsis* accessions and recorded how warmer and colder growth conditions affect the GSA trait variation. We subsequently used a genome-wide association study to identify single nucleotide polymorphisms in candidate genes that quantitatively impact the temperature-dependent setting of GSAs. These candidate genes will reveal further mechanistic insights into anti-gravitropic signal integration in LRs and hence root system architecture plasticity to ambient temperature.

O-73

Cytokinins Act Synergistically with Heat Acclimation to Enhance Rice Thermotolerance

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Heat stress is a frequent environmental constraint. Phytohormones, regulators of growth and stress responses, may significantly affect plant thermotolerance. This study compares the effects of exogenous cytokinin *meta*-topolin-9-(tetrahydropyran-2-yl)purine (mT9THP) on rice (*Oryza sativa*) at control conditions, after acclimation by moderate temperature (A; 37°C, 2h), heat stress (HS; 45°C, 6h) and their combination (AHS). mT9THP is a stable cytokinin derivative, gradually releasing active *meta*-topolin, which prevents fast deactivation reported after exogenous cytokinin application. Under control conditions, mT9THP negatively affected jasmonic acid in leaves and abscisic acid and salicylic acid in crowns (meristematic tissue crucial for tillering). Exogenous cytokinin stimulated emission of volatile organic compounds (VOC), especially 2,3-butanediol. Acclimation up-regulated endogenous cytokinin *trans*-zeatin, expression of stress- and hormone-related genes, and volatile emission. Combination of acclimation and mT9THP promoted expression of stress markers and antioxidant enzymes and moderately increased volatile emission, including 2-ethylhexyl salicylate or furanones. AHS and HS responses shared some features, namely increase in levels of ethylene precursor aminocyclopropane-1-carboxylic acid (ACC), *cis*-zeatin and cytokinin methylthio-derivatives, and expression of heat shock proteins, alternative oxidases and superoxide dismutases. AHS specifically induced levels of jasmonic acid and auxin indole-3-acetic acid, diacylglycerolipids with fewer double bonds, and massive volatile emissions [e.g., acetamide, lipoxygenase (LOX)-derived volatiles]. Under direct HS, exogenous cytokinin imitated some beneficial acclimation effects. Combination of mT9THP and AHS had the strongest thermo-protective effect, including vast stimulation of VOC emissions (including LOX-derived ones). The results demonstrated for the first time decisive contribution of volatiles to positive effects of cytokinin and AHS on rice thermotolerance.

POSTER PRESENTATIONS

*Posters marked with * are also presented as a Flash Talk.*

Biosynthesis and Metabolism

P-01-01

Does *Plasmodiophora brassicae* produce cytokinins?

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Many plant pathogens produce plant growth regulators to manipulate host development, physiology, and defence responses. Cytokinins (CK) can be produced by pathogenic bacteria, fungi and insects. It has been proposed that *Plasmodiophora brassicae*, the eukaryotic, obligate biotrophic pathogen responsible for clubroot disease of Brassicas, manipulates host developmental processes such as cell division, expansion, and differentiation by manipulating host CK homeostasis. However, the extent to which this occurs, and its importance in pathogenesis is unclear. The clubroot genome contains two isopentenyl transferase (IPT) genes which are expressed during gall formation. IPTs can produce CKs using adenine phosphates or tRNA as substrates. Adenine-IPTs productive active CKS. tRNA-IPTs produce *cis*-zeatin which is much less active in Brassicas. Also, tRNA-IPTs modify tRNA, which is important in protein synthesis. As a consequence, tRNA-IPTs are widespread and not necessarily involved in CK-induced changes in host development. In this study, we are experimentally characterising the clubroot IPTs to determine the importance of CK production in pathogenesis. The initial results show that both clubroot IPTs are tRNA-isopentenyl transferases. We will determine their importance in clubroot development and their role in host manipulation.

P-01-02

Multilevel characterization of acyl amido synthetases Gretchen Hagen 3 in tobacco BY-2 cells

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Auxin conjugation is one of the crucial metabolic processes regulating auxin activity in plant cells. Gretchen Hagen 3 (GH3) is a family of acyl amido synthetases that conjugates auxin with amino acids and include important players in maintenance of auxin homeostasis. GH3 enzymes are best known in thale cress (*Arabidopsis thaliana*), soybean (*Glycine max*), and rice (*Oryza sativa*), but detailed analysis of the reaction biochemistry in whole plant models is difficult. Therefore, we decided to investigate auxin metabolism in established model tobacco BY-2 cell line (*Nicotiana tabacum*). The *NtGH3.1* and *NtGH3.6* genes, which have been shown to vary in their expression regulation by auxin, were specifically mutated using the CRISPR/Cas9. In derived lines, auxin metabolic profiling was done by LC/MS and showed a decrease of metabolite oxIAA-Gln (N-(2-oxindole-3-acetyl)-glutamine) in *crntgh3.6d* line, which indicated a specific production of oxIAA-Gln by NtGH3.6d enzyme. RT-qPCR quantification of transcription in *crntgh3.1* and *crntgh3.6* lines indicated transcriptional compensation by upregulation of homologous *GH3* genes. Moreover, inducible expression of GFP-tagged tobacco GH3 members showed cytoplasmic and nuclear localization in tobacco BY-2 cells. Our results represent the first complete analysis of the expression, function, and localization of the individual members of the auxin acyl amido synthetases in tobacco.

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P-01-03

Quantitative analysis of phenylacetic acid conjugates in various plant species

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Auxins are group of phytohormones, which are essential for the plant growth and development. Several endogenous compounds belong to this group, such as indol-3-acetic acid (IAA), phenylacetic acid (PAA) and 4-chloroindole-acetic acid. Their proper function strictly depends on concentration gradients in plant organs and cells, which are maintained by processes that are still not fully understood, such as biosynthesis, transport, and conjugation. In most of the plants species PAA is more abundant than IAA, but concentration needed for auxin-like response induction is much higher. PAA metabolism pathways are similar to IAA pathways, as the same enzymes form the same conjugates, but so far, only three amino acids conjugates (PAA-Aspartate, PAA-Glutamate and PAA-Tryptophan) formed by group of GH3 enzymes have been identified in plants.

In our work, we developed a LC/MS-MS method for quantification of the whole PAA metabolite profile, identified and quantified three new amino acid metabolites PAA-Leucine, PAA-Phenylalanine and PAA-Valine, as well as PAA glucosyl ester in plants for the first time. Moreover, we quantified PAA metabolite profile in roots, shoots and cotyledons of Arabidopsis, pea, and wheat. We hope that these results will lead to better understanding of PAA metabolism and degradation, as these processes are still not fully explained.

P-01-04

Changes of the IAA catabolic network in auxin-starved BY-2 cells shown by computational modelling

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Auxin metabolism is a key regulatory process responsible, together with auxin transport, for the modulation of auxin concentration and hence also for the intensity of the respective hormonal signal. A wide spectrum of metabolites of the natural auxin IAA has been described, forming a complex network with numerous deactivation pathways. In order to elucidate how flexible this network is in its response to varying auxin availability, BY-2 tobacco cells grown in suspension with regular auxin-supplemented media as well as after a short period of auxin starvation were exogenously treated with IAA and the resulting metabolite levels were assessed using HPLC/MS in the cell contents and cultivation media. Subsequently, a mathematical model simulating the metabolic conversions together with mutual cell-media metabolite transport was developed in the MATLAB computing environment. This model was then fitted into the experimental data, thus obtaining estimates of the reaction rates from the kinetic parameters of the model. Comparison of these independent parameter estimates showed that auxin starvation resulted in a substantially decreased rate of both amino acid conjugation with IAA and the following oxidation of the conjugates on the one hand, with a simultaneous increase in the production of auxin decarboxylation metabolites indole-3-carbinol and oxindole-3-carbinol on the other. This shows considerable flexibility in the metabolic regulation of auxin levels, which thus needs to be considered in a number of physiological and developmental situations.

P-01-05*

Structural study on plant adenosine kinase

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Adenosine kinase (ADK) regulates the intracellular adenosine pool and catalyzes its phosphorylation to adenosine monophosphate using ATP as a cosubstrate. The major source of adenosine is the S-adenosyl methionine (SAM) cycle and adenosine has to be steadily removed by ADK to prevent the feedback inhibition of the SAM cycle and in consequence SAM-dependent transmethylation reactions and/or biosynthesis of ethylene or polyamines. In plants, ADK also contributes to the homeostasis of the plant hormone cytokinin. ADK is known to metabolize iPR or ZR and ADK-deficient plants have been shown to accumulate cytokinin ribosides. Moreover, ADK silencing impairs root growth, stamen and petal development, reduces meristem size, etc. To understand the structural basis of cytokinin conversion, five ADKs from maize (*Zea mays*) and moss (*Physcomitrella patens*) were analyzed. The purified ZmADK2 and ZmADK3 were crystalized and the X-ray structures were determined up to 2.05 Å resolution. The enzyme displays a two-domain topology and binding of the substrate induces a large rearrangement into a closed conformation. Finally, we prepared dexamethasone inducible *pOpON::ZmADK* lines in *Arabidopsis thaliana* to study *in vivo* metabolite changes.

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P-01-06*

Cytokinin Dehydrogenase Activity in Xylem Sap Reveals Direct Link Between Cytokinin Metabolism and Long-Distance Transport

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Cytokinin dehydrogenase (CKX) catalyses the cleavage of cytokinins, a major group of plant hormones. We performed a large-scale analysis of CKX proteins from various *Liliopsida* (monocots) species, which revealed a three-way correlation among CKX amino acid sequence, the identity of a previously described substrate-binding variable residue (here dubbed "VEGAS"), and the protein's predicted sub-cellular localization. These results portray CKX diversity as a widely conserved feature, allowing fine-tuning of cytokinin homeostasis maintenance. We further studied CKX specializations in a model plant of common oat (*Avena sativa*), an economically important crop. Quantifying *AsCKX* transcripts in different tissues revealed strong expression of extracellular forms in roots, suggesting their possible export to the xylem and degradation of cytokinins during their root-to-shoot transport. In our subsequent experiments, we detected CKX activity in oat xylem sap and demonstrated its dependence on the external availability of nitrate, a mineral source of nitrogen. Our findings suggest that CKX activity in the xylem is involved in cytokinin homeostasis modulation during long-distance transport and has a potential role in nitrate signalling.

P-01-07

Physiological effects of CK N-glucosides in plants

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Plant hormones cytokinins (CKs) regulate several growth and developmental processes in plants by activating intracellular signaling pathways. Although CK ribosides, ribotides and free bases of CKs are known to interact with CK receptors (Spíchal et al. 2004), only CK free bases have been considered as biologically active compounds in CK metabolism (Romanov and Schmülling 2021). In our study, we pursued glucose conjugates of CKs, CK *N7*- and *N9*-glucosides, both of which have long been characterized as irreversible storage forms of CKs without any relevant physiological effects in plants. Surprisingly, we observed different physiological effects of CK *N*-glucosides in various plant species, such as delay of leaf senescence, inhibition of root development, or alteration of gene expression after exogenous treatment. To elucidate these effects, we used different approaches also focusing on the *UGT76C1* and *UGT76C2* genes, both responsible for the formation of CK *N7*- and *N9*-glucosides. The results and observations we have obtained will be discussed at the conference.

Novel Methods and Techniques

P-02-01*

Boxeed: Precision Seeding and seed Phenotyping

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Many Arabidopsis research programs are focused on the detailed characterization of early developmental growth, followed by costly and laborious omics data processing. To make the most of such datasets, it is crucial to reduce any source of variability. One of the most challenging sources of variability is the micro-sized seeds and their manual handling.

Here, we introduce Boxeed, a state-of-the-art technology intended for non-invasive, dry-seed processing including phenotyping, sorting, counting as well as their precise seeding to growth media. Boxeed is designed for seeds between 80 µm – 3 mm, reaching average working speeds of 600 seeds/hour. Phenotyping is based on 2D image analysis of individual seeds from multiple projections. Seed morphometric and fluorescence parameters are calculated in real time, making it ideal for seed selection and seed sorting directly from stocks.

Using seed phenotyping and precise seed-to-seed positioning in *Arabidopsis thaliana*, we have identified significant variations in early postembryonic growth, which arise due to differences in seed size and seed-to-seed distance. Boxeed's seed sorting and precise seeding capabilities can reduce experimental variability thus enabling experimenters correlate hitherto hidden gene functions with phenotypes.

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P-02-02*

Cytokinin signal transduction: a systems biology approach

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The cytokinin signal transduction in plants involves a phosphorelay that activates a class of transcription factors, Response Regulators type-B (RRB). Besides activating their targets, RRBs also activate another class of Response Regulators type-A (RRA) that provide negative feedback in the network. Here we implement a systems biology approach to study the behaviour of this regulatory loop and explore putative interfaces for interactions with modulating pathways. For this, we adapted the ODE model from Cruz-Ramirez *et al.* (2012) and explored if this system would, or would not, show a bistable behaviour when simulating the different options for the interaction between RRB and RRA. We also investigate the effect of increased or decreased production of RRs on the behaviour of the system. We use TCS::GFP and ARR7::GFP fluorescent reporters to report on the behaviour of this network *in vivo* and test experimentally the predictions of our simulation. Our initial simulations indicate that our conceptual network does not show a bistable behaviour, implying it remains responsive towards minor changes in cytokinin concentrations over a wide concentration range. Furthermore, modulations of RRA production shift the sensitivity of the system towards cytokinins, highlighting the potential of RRAs to act as an interface for cross-talk between cytokinin signalling and other pathways. In summary, our research aims to combine systems biology and in vivo imaging to provide a framework for hypothesis generation and testing to integrate cytokinin signal transduction in plant development.

P-02-03

Engineered autonomous bioluminescence visualizes auxins and cytokinins

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Auxins and Cytokinins govern plant development and interactions with their natural habitat. The visualization of hormone distribution *in planta* with fluorescent proteins or biochemical assays is restricted to *in vitro* settings or snapshots of plant growth in soil. By engineering the biosynthesis of the fungal luciferin with constitutive promoters and the expression of luciferase under auxin- and cytokinin-specific promoters, we directed autonomous bioluminescence to reveal the distribution of these hormones in tobacco cell suspensions (BY2) or *Arabidopsis thaliana* growing in various conditions. Using simple, time-lapse photography with a commercial camera and lens, we record auxin- and cytokinin-sensitive bioluminescence *in vitro* to explore the time- and dose-dependent response by different auxins and cytokinins. Moreover, we reveal the distribution of these hormones in all organs of plants growing in near-to-nature conditions. Evidently, autonomous bioluminescence serves as a valuable method to explore auxin and cytokinin distribution and dynamics in stress conditions, such as drought, salinity, and adverse temperatures. In the future, we are interested in studying the effects of these stresses on the distribution and abundance of auxins and cytokinins of soil-grown *Arabidopsis thaliana* plants.

P-02-04*

Protoplast regeneration: the tools of gene transformation and foreign DNA-free gene editing

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Protoplasts are plant cells without cell walls. Protoplasts were first isolated in 1892 and enzymatic protoplast isolation was first applied to plants in 1960. Currently protoplast isolation, regeneration, transfection and transformation have been established in several crops and have been widely used in plant science research and crop breeding. Gene editing using cluster-regulated spaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas) is very convenient and can be performed with only a Cas protein and a single guide RNA (sgRNA) designed to target the sequence of interest. Genome editing reagents can be delivered by transfection into protoplasts that are edited and can be regenerated to foreign DNA-free plants. Here, we established a DNA-free CRISPR/Cas9 genome editing system that is based on optimized protoplast regeneration protocols for several crops. These protoplast regeneration techniques will greatly facilitate the domestication and breeding of crops.

P-02-05

Recent advances in plant hormones profiling

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Auxins are a group of plant hormones that affect a large part of the processes taking place in plant growth and development. The most important natural auxin indole-3-acetic acid (IAA) is involved in large part of the processes taking place in plant growth. The establishment and maintenance of auxin gradients within plant organs and tissues are coordinated by local IAA biosynthesis, metabolism, and transport. Interacting network of these mechanisms regulate auxin levels and distributions in plant tissues. This is important for driving of developmental stages or inducing appropriate responses to environmental changes. To improve our understanding of these mechanisms, information on levels of the free hormones as well as their metabolites is highly important. However, analysing plant hormones is demanding due to their very low concentrations and tremendous complexity of plant samples. A sensitive analytical method with the highest possible resolution is necessary for this purpose. Modern methods provide rapid and effective separation of several classes of phytohormones. Ultra-high performance liquid chromatography coupled with high-sensitivity tandem mass spectrometry (UHPLC-MS/MS) is the most widely used approach in phytohormone analysis. As conventional separation methods sometimes suffer from limitations in sensitivity and selectivity, there is a strong need for better separation techniques. We exploited recent advances in supercritical fluid chromatography (SFC) to take advantage of this highly efficient technique that overcomes the limits of other chromatographic methods. We have developed SFC-MS/MS method for determination of IAA metabolites and stress-related phytohormones that will be used for studying stress responses in various plant species.

P-02-06*

Chemical proteomic analysis of potential auxin molecular partners in *Arabidopsis thaliana* roots

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Auxins are fundamental phytohormones involved in a majority of plant physiological processes. A canonical mechanism of auxin signaling is based on the interaction of the activated TIR1 auxin receptor with AUX/IAAs repressors which are consequently submitted to ubiquitin-mediated degradation. To inspect other possible interaction partners of auxin, we employed a chemical proteomic technique using auxin-derived immobilized ligand as a bait. Thanks to the adopted approach, we were able to isolate a group of 50 proteins in addition to the expected TIR1 auxin receptor. Gene Ontology analysis of the detected proteins supported their relevance for the biological processes influenced by auxins.

P-02-07

Novel HILIC-MS/MS method for separation and quantification of all isomers of cytokinin glucosides

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Hormonal homeostasis in a given plant tissues is tightly regulated during plant growth, development and response to environmental cues. This is achieved by regulating biosynthesis, interconversion, transport and by regulation of catabolism. In the case of cytokinins, free bases, the most biologically active forms, could be catabolised by the action of cytokinin oxidase/dehydrogenase or conjugated with glucose at the N7 and N9 positions, and zeatin-type cytokinins can additionally undergo *O*-glucosylation. However, there is an increasing evidence that each of these isomers could have different role in plants. This creates a problem. As to confidently test experimental hypotheses, researchers therefore require method selective enough to correctly quantify these chemical species individually. From the analytical point of view, this is rather difficult, and especially in the case of a separation of all *cis*- and *trans*- zeatin glucosides. Mass spectrometry detection systems are not capable to selectively distinguish between these chemical species, and thus complete chromatographic separation is required. We have successfully developed a selective method capable of quantification of all the zeatin glucosides isomers using hydrophilic interaction chromatography with sensitive mass spectrometry detection. This bypasses the need of laborious and time consuming sample preparation that is based on immunoaffinity extraction and subsequent enzymatic cleavage. This newly developed method enables a better, faster, and easier way to confidently quantify cytokinin glucoside isomers in plant tissues that could be utilized in studies potentially unraveling biological roles of these compounds.

Transport

P-03-01

Epigenetic Regulation of PIN1

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The auxin efflux carrier PIN is the key mediator for polar auxin transport in developing plant tissues. To establish polar auxin transport, a narrow PIN-positive channel needs to be established. This coordination requires an intricate interplay of many proteins. The PIN expression itself is regulated by auxin, however, we have a very limited understanding of how mechanistically it is manifested. Several studies have characterized a long non-coding RNA, APOLO, that plays a role in this process. The APOLO lncRNA functions as a scaffolding RNA and takes a role in the creation of chromatin loops. APOLO uses chromatin folding, loop creation, and promoter methylation to mediate the silencing of PINOID (PID). *PID* is the auxin-responsive gene, and it is essential for proper cotyledon positioning and development, for maintenance of the inflorescence meristem, for whorl definition during flower development and it is important for wild-type root growth.

A novel long non-coding RNA (lncRNA) was identified within the PIN1 promoter region. Our investigation of their function in the PIN1 promoter revealed significant differences between transcriptional reporter pPIN1::NLS-GFP-GUS transgenic line with and without lncRNA deletion. Notably, when the long non-coding RNA (lncRNA) is absent, PIN1 expression is significantly upregulated in the leaves and primary roots, but not in the lateral roots. This tissue-specific epigenetic mechanism could shed new light on the non-coding fine-tuning of PIN1 expression in the development of distinct plant tissues.

Keywords: lncRNA, PIN1, Auxin

P-03-02*

EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1: A new cytokinin transporter

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During the last decades, we gained considerable knowledge on cytokinin's chemical nature, biosynthesis, signal perception, and physiological role in plant development and growth. Yet, their transport is poorly characterized. A model of the cytokinin long-distance distribution was introduced with Arabidopsis ABCG14 transporter playing a crucial role. However, it would not be surprising if also certain members of the equilibrative nucleoside transporter (ENT) family, played a role in long-distance cytokinin transport.

By the combination of analytical chemistry and molecular biology methods, we found a new cytokinin transporter of the ENT family. For the first time, we were able to observe *in planta* ENT1 localization by confocal microscopy. Our results indicated localization at the plasma membrane, and the tonoplast in the epidermal cells of the root apical meristem. Using a transgenic inducible system in tobacco BY-2 cells demonstrated that ENT1 is capable of specific cytokinin transport, and experiment with cytokinin tracers showed translocation both at the subcellular level (vacuoles) and at the tissue level. Additionally, our initial findings utilizing *pENT1::GFP-GUS* lines reveal vascular expression of ENT1 that was not documented in the previously published data. The observed results, in combination with ENT1's demonstrated ability to transport CKs at the cellular level, indicate the potential for AtENT1 to function as a long-range cytokinin transporter.

These results suggest an interesting yet unspecified role of ENT1 in cytokinin transport. Further investigations will help us expand our current view on these important plant hormones.

P-03-03*

Uncovering intramolecular dynamics in the auxin transporter ABCB1 using genetically encoded activity sensors

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Plant development and performance is highly dependent on the precise cellular distribution of the plant hormone auxin. This is provided by different classes of auxin transporters, with the B family of ABC transporters (ABCBs) acting as auxin pumps. To date, several tools, including genetically encoded auxin reporters and sensors, have been used to monitor auxin flux, but these have the drawback of integrating over transport and homeostasis. Here, we have designed and tested a series of genetically encoded ratiometric sensors of ABCB activity by fusing circularly permuted GFP (cpGFP) at eight different positions and an RFP at the C-terminus of ABCB1, allowing monitoring of protein movement by changes in fluorescence intensity. *In vitro* and *in vivo* experiments showed that the native and synthetic auxins, indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA), respectively, caused opposite changes in the cpGFP/RFP fluorescence ratio in most variants. This suggests that although both auxins are transported by ABCB1 and are thought to bind to the same substrate binding domain, they have different effects on the conformation of the transporter. To understand this unexpected result, we performed a series of *in silico* dockings of IAA and NAA to the central cavity of ABCB1, which showed that IAA and NAA use widely different contact sites, providing a reasonable relationship for opposite cpGFP/RFP fluorescence ratios. These results demonstrate the potential of genetically encoded ABCB activity sensors to provide valuable information on the intramolecular dynamics of transporters *in vivo.* This approach holds great promise for imaging ABCB-mediated auxin fluxes *in planta.*

P-03-04

Transport-based mechanisms for the auxin-autonomous proliferation of plant cells

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The ability of habituated plant cell lines to proliferate in the absence of exogenously added plant hormones (auxins, cytokinins) has been known for more than fifty years. However, the underlying mechanisms still remain elusive. To address this, we have thoroughly analyzed RNA-seq profiles from two auxin-dependent (BY-2, VBI-0) and two auxin-autonomous (BY-2H, VBI-2b) tobacco cell lines. We have identified a set of differentially expressed genes that could be responsible for auxin autonomy. Our focus was on genes belonging to two gene ontology terms: response to auxin and auxin-activated signaling pathway to which the genes responsible for auxin biosynthesis, metabolism, and transport were added. In the following work, by using auxin transport assays in auxin-dependent and auxin-autonomous lines, we show that in both BY-2H and VBI-2b, the auxin carrier-mediated auxin efflux and influx are dramatically decreased. We have correlated these differences with auxin metabolic changes to obtain a detailed view of the auxin homeostasis in tobacco cells. Our results support the hypothesis that fine-tuning of carrier-mediated auxin transport is involved in the maintenance of endogenous auxin levels sufficient for cell proliferation.

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P-03-05

Investigation of the role of equilibrative nucleoside transporters in cytokinin transport

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Cytokinins (CKs) are *N6*-substituted derivatives of adenine found in various organisms. In plants, they play a central role in vascular development, organogenic response, lateral root morphogenesis, and general shoot development. In addition, CKs act in response to stress and as a mobile signal for mineral availability. As such long-range signals, CKs are transported through the vasculature, usually in the form of ribosides (nucleosides) or nucleotides. Conjugation with the ribosyl group increases the molecular polarity and bulkiness of CKs. Both prevent CKs from escaping from the vasculature by simple diffusion, suggesting the need for a transmembrane carrier. Plant equilibrative nucleoside transporters (ENTs) are suitable candidates for such a role, as some members of this family have shown affinity for CK ribosides in heterologous biochemical assays. In addition, plants carrying *ent* loss-of-function mutations have shown CK-related deficiencies.

To investigate the role of ENTs in cytokinin transport in the context of plant cells, we use tobacco BY-2 cells grown in suspension culture. Feeding experiments with tritiated *trans*-zeatin riboside and isopentenyladenosine in BY-2 lines overexpressing *AtENT1* and *AtENT3* under an inducible promoter show an increased influx of both compounds. Our results suggest that certain ENTs indeed promote the transport of CK ribosides across the plasma membrane of plant cells.

P-03-06

Evolutionary divergences in PIN-mediated auxin transport

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Canonical auxin signaling and biosynthesis only emerged in land plants, but PIN auxin transporters are present in the closely related streptophyte green algae. This poster shows the convoluted evolution of PINs in these organisms. Some lost PINs altogether, others like stoneworts expanded the family. The early-diverging Klebsormidium retained functional PIN-mediated auxin transport. In the algae most closely relanted to land plants, however, PINs do not appear to transport auxin, as we show here. These patterns are discussed in the context of available auxin-related knowledge in green algae and the evolutionary changes in their morphology.

P-03-07

Functional characterization of the cytokinin binding protein in cytokinin regulated plant growth and development

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Cytokinin is a plant hormone that regulates plant growth and development. Although the canonical cytokinin pathway is well established, cytokinin has some regulations independent of the canonical pathway. For example, cytokinin-mediated auxin efflux carrier PIN1 degradation depends on the cytokinin receptors but is independent of transcription and translation. However, how cytokinin bypasses the transcription is unknown. To understand cytokinin's non-canonical roles, we performed affinity-purification of cytokinin-binding proteins (CBP) from Arabidopsis thaliana root protein lysate. The dynamin-related protein was recognized among the top CBP candidates. The cytokinin binding to the candidate CBP was confirmed using cellular thermal shift assay (CETSA) and Drug Affinity Responsive Target Stability (DARTS). Functional characterization of the CBP in cytokinin-regulated plant development will be discussed.

P-03-08*

A direct, regulatory circuit between the LRR receptor kinase, ALK1/QSK1/KIN7, and ABCG36/PEN3/PDR8 controls transporter substrate preferences during plant growth and defense decisions

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Plants have evolved refined mechanisms to balance growth-defense tradeoffs. Based on its proposed substrate preferences, the Arabidopsis ABC transporter, ABCG36/PEN3/PDR8, stands at the interface between growth and defence: ABCG36 was shown to export the auxin precursor, indole-3-butyric acid (IBA), and to be implicated in the export of the major phytoalexin of Arabidopsis, camalexin.

Here we provide strong evidence that ABCG36 catalyses the direct, ATP-dependent export of camalexin over the plasma membrane. We identify the leucin-rich repeat receptor kinase, Auxin-induced LRR Kinase1, ALK1/QSK1/KIN7, as a functional kinase to physically interact with and phosphorylate ABCG36. ABCG36 phosphorylation by ALK1 represses unilaterally IBA but not camalexin export leading to a prioritization of ABCG transport toward defense. As a consequence, phospho-dead mutants of ABCG36, like *alk1* and *abcg36* alleles, are hypersensitive toward infection with the root pathogen, *F. oxysporum*, caused by elevated fungal progression.

Our findings indicate a novel, direct regulatory circuit between a receptor kinase and an ABC transporter determining transporter substrate specificity.

Signalling

P-04-01

Evolutionary origin of the auxin signaling pathway

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The question of which innovations played a crucial role in the transition of plants to terrestrial environments, and how they evolved, remains largely unexplored in plant biology. Our research specifically focuses on the origin of auxin biology and aims to reveal the molecular and cellular mechanisms that respond to auxin in streptophyte algae, the sister lineage to land plants.

Phylogenetic analysis has provided evidence that some components of the plant auxin response system are present in this algae, indicating that the system may have been present in a common ancestor. However, our understanding of this topic is mostly based on genomic and transcriptomic information. To expand our knowledge, we conducted experiments on *Penium margaritaceum*, a type of green algae, using transcriptomics, phosphoproteomics, and microfluidics to investigate auxin responses.

P-04-02

Concentration is key: unravelling mechanisms controlling auxin response factor stability

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The molecule auxin controls nearly all developmental processes in land plants through the nuclear auxin signalling pathway (NAP). The NAP consists of an auxin receptor TIR1/AFB, its AUX/IAA degradation substrate, and the DNA-binding ARF transcription factors. While an extensive qualitative understanding of the pathway and its interactions has been obtained by studying the flowering plant *Arabidopsis thaliana*, it is so far unknown how these translate to quantitative system behaviour *in vivo*, a problem that is confounded by large NAP gene families in this species. Here, we exploited the minimal NAP of the liverwort *Marchantia polymorpha* to quantitatively map NAP protein accumulation patterns and their dynamics *in vivo* using knock-in fluorescent fusion proteins. Beyond revealing the native accumulation profile of the entire NAP protein network, we discovered that the two central ARFs, MpARF1 and MpARF2, are degraded via the proteasome. We mapped the degrons of both MpARFs to specific domains and show that MpARF2 dimerization is an important factor for degron accessibility and thus protein stability. In future work we aim to fully uncover the molecular mechanisms which define MpARF stability through a variety of approaches which will allow us to study functional implications of targeted MpARF proteolysis for plant development.

P-04-03*

Exploring the spatial organisation of cytokinin action to regulate size and activity of the shoot apical meristem

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Plants initiate new organs throughout their life cycle. The organs of the shoot are produced by the shoot apical meristem (SAM). In its centre, the SAM maintains a group of continuously dividing stem cells (SC) that provide cells for the meristem and forming organs. A complex network of genetic factors and plant hormones controls the dynamic balance of cell division, maintenance and differentiation of the SC thus determining SAM size and activity. Cytokinin (CK) is a positive regulator of the SAM acting at least partially through the WUS/CLV pathway. However, the spatial organization of CK action is not well understood. To explore the functional relevance of CK in different domains of the SAM we increased or lowered the CK content or signaling in distinct meristematic domains. The constitutively active cytokinin receptor variant ROCK2, activating or silencing variants of the B-type response regulator ARR10, or a cytokinin-degrading CKX enzyme were expressed under control of the pFBDl, pCLV1, pWUS and pCLV3 promoters in wild type as well as in B-type arr mutants. The results show that enhanced cytokinin degradation in the CLV1 domain is sufficient to reduce SAM size. ROCK2 expression in the stem cells retards the transition from cell division to differentiation, thus causing a drastic increase of SAM size. The altered CK activity may change additional parameters of growth and development, such as rosette size, stem diameter, and the duration of flowering time. We will report on the ongoing analysis of transgenic lines with altered CK status and their phenotypes.

P-04-04*

Modulation of PIN2 by TRANSMEMBRANE KINASE 1 for robust root gravitropism

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Plants utilize directional auxin transport through PIN auxin efflux carriers to effect various adaptive responses to their environment as well as to develop from seed to seed. Classic observations on the establishment of vascular strands led to the canalization hypothesis whereby auxin directs and feeds back onto its own flow. We recently identified components of cell-surface auxin signalling as upstream of PIN2-mediated fluxes during root gravitropism. Genetics, imaging and biochemical data show that TRANSMEMBRANE KINASE 1 (TMK1) directly interacts with and phosphorylates PIN2, thus modulating its levels, which is required for initial stages of gravitropic root bending. Nevertheless, the exact molecular logic behind TMK1 effect on PIN2 remains unclear. To address this problem, we manipulated ABP1-TMK1-dependent phosphorylation sites on PIN2 to either mimic or prevent their phosphorylation. Altogether, TMK1 interaction with and phosphorylation of PIN proteins provides a parsimonious mechanism for immediate auxin feedback on its transport. This is relevant both for rheostatic modulation of PIN stability during gravitropism (presented here) and for polarity regulations during auxin canalization.

P-04-05*

Resolving regulation of autophagy by auxin

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Auxin plays a vital role in regulating various biological processes, ranging from embryogenesis and organogenesis at a macroscopic level, to specific cellular activities like ion exchange, cell polarity, and endocytic trafficking (Sauer et al., 2013). Additionally, recent research has shown that auxin is possibly involved in the activation of another crucial cellular process that is macroautophagy (Rodriguez et al., 2020).

Macroautophagy (from now on referred as autophagy) is a “self-eating” catabolic mechanism for the removal of unneeded or dysfunctional cytoplasmic contents, such as protein aggregates or damaged organelles (Gou et al., 2019).

Although autophagy was initially thought of as a coping mechanism for damage and different stresses, there is now proof that this process is essential for cell homeostasis, particularly in the short-term reprogramming of somatic cells. In order to enable quick changes in cell state, autophagy thus appears to be highly controlled by a variety of pathways that are convergent on it. (Batoko et al., 2017; Rodriguez et al., 2020). Here, we will present how auxin can fine-tune autophagy to rewire the somatic cell state, using characterized auxin analogues, genetic methods and proteomics approaches.

P-04-06

Distinct functions of TIR1 and AFB1 receptors in auxin signalling

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Auxin is the major plant hormone regulating growth and development (Friml, 2022). Forward genetic approaches in the model plant *Arabidopsis thaliana* have identified major components of auxin signalling and established the canonical mechanism mediating transcriptional and thus developmental reprogramming. In this textbook view, TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN-SIGNALING F-BOX (AFBs) are auxin receptors that act as F-box subunits, determining the substrate specificity of Skp1-Cullin1-F-box (SCF) type E3 ubiquitin ligase complexes. Auxin acts as a "molecular glue" and increases the affinity between TIR1/AFBs and the Aux/IAA repressors. Subsequently, Aux/IAAs are ubiquitinated and degraded, thus releasing auxin transcription factors from repression and making them free to mediate the transcription of auxin-responsive genes (Yu *et al.*, 2022). Accumulating evidence suggests the existence of rapid, non-transcriptional responses downstream of TIR1/AFBs such as auxin-induced cytosolic Ca^{2+} transients, plasma membrane depolarization, and apoplast alkalinization, all converging on the process of root growth inhibition and root gravitropism (Li *et al.*, 2022). These rapid responses are mostly contributed by the predominantly cytosolic AFB1, while the long-term growth responses are mediated by mainly nuclear TIR1 and AFB2-AFB5 (Li *et al.*, 2021; Prigge *et al.*, 2020; Serre *et al.*, 2021). How AFB1 executes auxin-triggered rapid responses and how it is different from TIR1 and AFB2-AFB5 remains elusive. Here we compare the roles of TIR1 and AFB1 in transcriptional and rapid responses by modulating their subcellular localization and testing their ability to mediate transcriptional responses in yeast.

P-04-07

Ethylene biosynthesis is differently regulated in spatial-temporal manner

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Rice is one of the most important crop. Ethylene, a plant hormone, affects development, growth, and response to various internal and external stresses in plants, including rice. Ethylene biosynthesis is tightly regulated by external and internal cues. Previously, we demonstrated that various phytohormones, including auxin and cytokinin, affect ethylene biosynthesis by transcriptional and/or post-transcriptional regulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthases (ACS) and ACC oxidases (ACO) in etiolated Arabidopsis and rice seedlings. However, until now, it is not known how *OsACS* genes is expressed differently in each tissue and at each stage of rice development. Here, we report on the temporally-spatially differentially expressed *OsACS* genes. Except for *OsACS2* in 2-week-old rice, it is most expressed in roots and leaf blades. *OsACS2* was most frequently expressed only in the leaf blade. In 12-week-old rice, *OsACSs* were highly expressed in leaf blades, but also in pistile and stamen. In 14-week-old rice, expression of *OsACSs* were low in milk grain. It was confirmed that each *OsACS* gene was differently expressed during the growth period. Our study identified *OsACS* genes that are differently expressed in rice growth and each tissue, which will provide an important basis for developmental studies.

P-04-08

Vasculature development mediated by auxin canalization in Arabidopsis inflorescence stems

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Vasculature development around the wound during regeneration is developmentally fascinating process that leads to coordinated polarization of cells and auxin canalization. The classical canalization hypothesis proposes that the plant hormone auxin acts as the polarizing signal, which by means of a positive feedback between auxin perception and its intercellular transport mediate auxin channel formation for vascularization. Using mechanically stimulated inflorescence stems of *Arabidopsis thaliana* we established an experimental system capable of vasculature regeneration after its mechanical interruption and vasculature formation from the local external auxin source. This gives a unique opportunity to approach genetically still open questions related to the auxin canalization hypothesis.

The key process in auxin canalization – formation of polarized auxin channels away from the localized auxin source is conceptually unclear. How is the auxin signal propagated across the tissue and how does it allow for coordinated polarization of individual cells? Our observations provide novel insights regarding the cellular mechanisms underlying auxin canalization-mediated vascular tissue formation. Directional auxin flow depends on the asymmetric, plasma membrane position of the PIN auxin exporters protein in transporting cells. The impact of auxin on the polar PIN1 positioning is likely a part of a mechanism for auxin canalization. In this view, local auxin accumulation above the wound would simultaneously trigger the TIR1/AFB-mediated intracellular and ABP1/TMK1 cell surface signalling pathways ultimately determining *PIN* expression and PIN polarity for formation of auxin-transporting channels. This global auxin-mediated coordination acts in concert with additional perception system dependent of CAMEL and CANAR receptor kinases complex.

P-04-09*

The receptor kinase LRR6 as a player in auxin canalization

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The phytohormone auxin is unique due to its ability to polarize its own transport in the plant tissues; this is achieved by the establishment of auxin channels from its localized source. This self-organizing process, so called auxin canalization, provides a positional information for the formation of the vasculature during plant development, for instance during the leaf venation and the regeneration after wounding. It involves the feed-back regulation between auxin signaling and transport. Recently, it has been shown that the cell-surface auxin signaling complex, consisting of AUXIN BINDING PROTEIN1 (ABP1) and TRANSMEMBRANE KINASE1 (TMK1), plays an essential role in auxin canalization. Despite the formulation of the canalization hypothesis decades ago, the underlying mechanism is still not fully resolved on the molecular level and requires more investigations and identifications of additional molecular players and their relationships. Here we present the leucine-rich repeat receptor-like kinase6 (LRR6), which interacts with TMK1 and its knockout mutant shows defect in the vasculature regeneration after wounding. Therefore, we explore its involvement along with other interactors in ABP1/TMK-mediated auxin canalization.

P-04-10

Role of 4-Chloroindole-3-acetic Acid in Promoting Pea Pod Elongation

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The plant hormone auxin influences almost every aspect of plant growth and development. There are five naturally occurring auxin in plants namely, indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), phenylacetic acid, indole-3-butyric acid (IBA), and indole-3-propionic acid (IPA). Peas, along with other species in the Fabeae and Trifolieae clades of Fabaceae produce the chlorinated variant of auxin, where a chlorine atom covalently attaches at the 4′ position of its indole ring. This 4-Cl-IAA has been implicated to have role in fruit development and seed starch metabolism. However, the exact mechanism of action is unknown. Using a combination of molecular and genetic tools, we aim to investigate 4-Cl-IAA signalling and its transport events that control pea fruit development. Preliminary results will be reported.

P-04-11

Dissecting ubiquitin-ligase and adenylyl-cyclase activity of TIR1

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Auxin signaling has recently extended from the canonical TIR1/AFB-Aux/IAA-ARF pathway to a more complex network leading to both transcriptional and non-transcriptional responses. The TIR1/AFB auxin receptors have both ubiquitin-ligase and adenylyl-cyclase activities. This makes Aux/IAA degradation and cAMP production two distinct outputs of auxin perception. It requires further clarification on how much these two distinct mechanisms participate in the transcriptional auxin response. We addressed this question by using both a minimal yeast system and a more complex *in planta* protoplast system.

Yeasts contain endogenous E3 ubiquitin-ligase complexes. Transformation with elements of auxin signaling as TIR1, Aux/IAA or ARFs is sufficient to reconstitute a functional auxin sensing pathway. To test if the ubiquitin-ligase and/or the adenylyl-cyclase activity are required for auxin response, we mutated TIR1 either in the adenylyl-cyclase domain or in the F-box domain, which is required for Aux/IAA degradation.

To assess the effect of TIR1 mutations over a broad range of conditions, we used a method based on classical protoplast transformation. The transient PEG transformation produces a cell population with variable expression of transgenes. We used this variability to quantitatively test the effect of protein levels on the response. Implementing an automatic image analysis pipeline, we multiplexed the experiments to a wall plate format. By scaling up the experiments, we managed to assess auxin signaling in time and combined ranges of protein expression and IAA concentrations.

These approaches will contribute to clarifying the importance of the TIR1/AFB ubiquitin-ligase and adenylyl-cyclase activities for canonical auxin response.

P-04-12

Decoding ARF DNA-binding: MpARF1-His146's potential role in DNA-binding

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Auxin Response Factors, the key transcription factor family of the nuclear auxin pathway, is known to bind TGTCNN motifs (where N is any base). A single histidine residue, H146 in *Marchantia polymorpha* (Marchantia) ARF1 (MpARF1), binds to the final two nucleotides of the motif and decides whether an element is a high affinity motif (TGTCGG) or medium affinity (TGTCTC) motif. On an atomic level it performs this function via a subtle alosteric rotamer change. Here, we asked whether natural variation for this residue could alter the DNA-binding preferences of an ARF, and subsequently could alter growth and development. We used a complementation assay with a null mutant of the sole activator ARF in Marchantia (MpARF1) and found that H146N and H146Y mutants had an altered size compared to the wild type strain Tak-1. We found that both mutants had lowered binding to TGTCGG motifs, but that this can be compensated *in planta* by higher expression levels of MpARF1-H146N. To conclude we found that altering a single residue can subtly alter DNA-binding and affect plant growth.

P-04-13*

Auxin and its feedback signalling on PILS turnover

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The phytohormone auxin is a key regulator of many biological processes. It plays a central role in cell division, elongation and differentiation and helps the plants adapting their growth in response to environmental (Feraru et al., 2019) as well as internal stimuli (Sun et al., 2020). Auxin spatiotemporal distribution in the tissues is regulated through metabolism and transport, but while intercellular transport has been deeply investigated, little is known about auxin subcellular transport. The PIN-LIKES (PILS) is a family of intracellular auxin transport facilitators, that control auxin accumulation in the endoplasmic reticulum, reducing auxin availability and signalling in the nucleus.The molecular mechanisms regulating PILS turnover are still not clear, but our recent data suggests that the ER-Associated Degradation (ERAD) machinery plays a central role in this process, tagging auxin for proteasomal degradation. We moreover recently showed that PILS abundance is controlled by auxin availability (Feraru et al., 2022). Here we will discuss a transceptor-like (transporter and receptor) function of PILS proteins, by addressing whether auxin binding to PILS directly defines the degradation rate of PILS proteins.

P-04-14*

In-silico modelling of ABA, SnRK2, SnRK1 and TOR signalling pathways

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Despite the negative impact of stress on crop productivity, how growth is modified by stress signaling pathways is poorly understood and some parts in these pathways are completely obscured. Innovative systems biology tools allow bioinformatics to model different situations and thus reveal the correct patterns reflected in life science. In this study, we focus on the implementation of biological knowledge to extend signaling pathway databases to the plant kingdom. Therefore, we focus on the ABA signalling pathway in Arabidopsis thaliana. The phytohormone ABA plays crucial roles in numerous physiological processes during plant growth and abiotic/biotic stress responses. Endogenous ABA levels are involved in complex regulatory mechanisms in signal transduction pathways and show interwoven network relations to sucrose non-fermenting 1-related protein kinase 2 (SnRK2) but also SnRK1 signalling pathways. The core ABA signalling pathway is composed of receptors in the pyrabatin resistance/pyrabitin resistance-like/regulatory components of ABA receptor (PYR/PYL/RCAR) family, type 2C protein phosphatases (PP2Cs), sucrose non-fermenting 1-related protein kinase 2 (SnRK2s) and substrates of SnRK2s. If ABA is present, the receptor RCAR/PYR/PYLs binds to PP2Cs, inhibits the enzymatic activity of PP2Cs and simultaneously dissociates the PP2Cs-SnRK2s complex. During the positive feedback loop we found two attractors representing stable states of the signalling pathways. In contrast, modelling with negative feedback loop showed only one possible attractor and one chaotic situation. We simulated this model using different tools and at the end we also add information about the evolutionarily highly conserved energy-sensing SNF1-related protein kinases 1 (SnRK1) and its interaction with TOR signalling.

P-04-15*

Loss of auxin signalling components in duckweed is linked to a loss of body plan complexity

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Duckweed is a family of small aquatic plants that are found all over the world, made of five genera. The genus that best represents the ancestral form, *Spirodela*, grows roots. The most recently derived genus, *Wolffia*, does not grow roots and lacks vasculature. The loss of body plan complexity during duckweed evolution is startling, akin to the loss of limbs in snakes. Auxin is essential to many facets of plant development, including organogenesis. In *Arabidopsis thaliana*, AUXIN RESPONSE FACTOR 5/MONOPTEROS (ARF5/MP) is essential for root pole establishment during embryonic development. Mutation of ARF5/MP leads to loss of root development and disrupted vasculature in *Arabidopsis* embryos. The ancestral, rooted duckweed *Spirodela polyrhiza*, has an ARF5/MP orthologue, but the recently derived, rootless *Wolffia australiana* doesn't. We hypothesise that in duckweeds, loss of ARF5/MP and other signalling components is linked to the loss of body plan complexity. Hormone treatments show that *W. australiana* is less sensitive to changes in auxin than *S. polyrhiza*. Bioinformatic analyses show that there has been significant loss of auxin signalling components across the duckweeds, most strikingly in the AUXIN/INDOLE-3 ACETIC ACID family, as well as a loss of cytokinin signalling components. We are undertaking transcriptome sequencing following auxin treatment of three duckweed species and two model monocots to couple the complexity of auxin signalling networks with response kinetics. Following this, I will transform the *Spirodela* ARF5/MP orthologue into *Wolffia* and knockdown/knock-out ARF5/MP in *Spirodela*, to functionally dissect physiological and gene network responses to additional/removal of this key regulator.

P-04-16

The mechanism of auxin regulation of stomata movement

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Stomata are tiny pores found in the surface of plant stems and leaves. They play crucial roles in the exchange of gas and water vapor between the plant and environment. The opening and closing of the stomata are regulated by complex signaling pathways in respond to various internal and external stimuli. Each stoma is surrounded by two specialized epidermal guard cells. The turgor pressure of guard cell is a fundamental feature that controls the movement of stomata. The activation of proton export out of the guard cells increases the membrane potential and proton gradient. This promotes the influx of potassium ion which triggers water uptake and cell swell, leading to the opening of stomata. The phytohormone auxin activates plasma-membrane H^+-ATPase and potassium ion channels to regulate stomata movement in a dose dependent manner. However, the mechanism of auxin regulation of stomata movement is not fully understood. Auxin, as a signaling molecule, regulates plant growth and development by transcriptional and non-transcriptional pathways. We aim to establish a system to study auxin-induced stomata movement in *Arabidopsis*, and to explore the mechanism of Auxin Binding Protein 1 (ABP1), and Transmembrane Kinase 1 (TMK1) – H^+-ATPase (AHA) signaling in stomata movement.

P-04-17

Elucidation of the biological activity of new auxin derivatives in *Arabidopsis thaliana*

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Auxin is one of the major phytohormones, which controls various aspects of plant growth and development. Establishment of auxin concentration gradients, collectively mediated by auxin biosynthesis, metabolism and polar transport, determines, among others, plant organ positioning and growth responses to environmental stimuli.

Here we report novel auxin derivatives. These compounds do not possess auxin activity but, on the contrary, they inhibit auxin-induced responses. The most active derivatives showed strong anti-auxin activity in roots and hypocotyls, which also occurred at the gene transcription level as confirmed by quantitative PCR analysis. Moreover, the auxin antagonism of our derivatives was also confirmed *in vitro* by SPR-based binding assay.

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Development

P-05-01

The role of HIPP proteins in the regulation of lateral root development

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HEAVY METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEINS (HIPPs) have emerged as novel regulators of growth and development in vascular plants. However, the molecular mechanisms underlying their activity are currently completely unknown. Our research revealed that cluster III HIPP proteins from Arabidopsis control root system architecture by negatively regulating lateral root development. The genetic data are consistent with a strong expression of *pHIPP:GFP* transcriptional reporter lines in lateral root primordia. To further investigate the genetic and molecular pathways underlying the function of the HIPP proteins, yeast two-hybrid analysis was employed. Interestingly, specific interactions between HIPPs and several members of the AUXIN RESPONSE FACTOR (ARF) family were identified. The HIPP-ARF interactions were validated *in planta* and the protein domains involved in these interactions mapped. This research project aims to understand whether the HIPP-ARF protein-protein interactions affect the ARF activity and to determine the function of these interactions during lateral root formation.

P-05-02

PHD-HD proteins: an enigmatic plant-specific transcription factor family

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The PHD-HD family is a plant-specific transcription factor (TF) family that combines a plant homeodomain (PHD) and homeodomain (HD) architecture. Notably, HAT3.1 and PRHA are the only members of the PHD-HD family in *Arabidopsis* whose molecular or physiological function is yet to be fully understood.

We analyzed the subcellular localization of HAT3.1 and confirmed its nuclear targeting. Interestingly, ectopically expressed HAT3.1-eGFP protein exhibits a speckled nuclear distribution, implying its yet unknown functional compartmentalization within the nucleus. Moreover, the investigation of the *HAT3.1* expression pattern using a *proHAT3.1::EGFP-GUS* reporter line revealed its strong association with the regions of actively dividing cells in both vegetative and generative tissues.

As plant development is regulated by both intrinsic and exogenous factors, we analyzed the responsiveness of *HAT3.1* expression to various stimuli. We found that auxin positively regulates *HAT3.1* expression, likely due to the auxin-induced initiation of lateral roots and callus formation. Conversely, salt stress not only reduces *HAT3.1* promoter activity but also affects the root length and germination rate of *hat3.1* and *prha* mutant line, as shown by our preliminary phenotyping.

Our findings suggest that PHD-HD proteins are involved in early plant developmental processes, and their function can be influenced by exogenous factors, such as salt stress. Further studies on this unique TF family could provide insight into novel regulators of plant growth and development.

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P-05-03

Impact of PAT damage on the vascular trace development in Arabidopsis inflorescence shoots

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The development of the conducting system in the shoot is closely related to organogenesis. With the emergence of a new organ such as a leaf, flower or axillary bud, the new vascular strands (traces) develop to connect this organ with the existing conducting system. This ensures integration of the newly formed organ with the entire plant. As previous studies on *Arabidopsis thaliana* indicated, the development of the vascular trace is a multi-stage process, regulated by PIN1-dependent PAT that determines the place of new trace formation and induces its development.

In our study, we followed the successive stages of the vascular trace formation, induced in *pin1* mutant by the application of exogenous auxin at the SAM. We used fluorescent markers for the preprocambium and procambium stages, phloem staining methods and 3D visualization of the xylem strands and found that PIN1-dependent PAT is not required for any of the stages of the vascular trace development, nor for the formation of vascular connections. However, PAT, if present, modifies the preprocambium stage, changing the spatial pattern of the entire conducting system, and the xylem strands development, increasing the efficiency of their differentiation. Importantly, impairment of the PIN1-dependent PAT seems to be irrelevant to the procambium development and phloem strands differentiation, suggesting that the transport of auxin necessary for these both processes is only PAT-independent.

P-05-04

The role of auxin and cytokinin in vascular reconnection during graft formation

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Auxin and cytokinin are known to play an important role in the process of vascular reconnection during graft junction formation. While most plant hormones can be implicated in vascular differentiation, auxin is understood to be the core regulator. Auxin is predominantly produced in the shoot and moves basipetally through the stem. During the initial stages of graft formation, auxin accumulates asymmetrically, with higher concentrations above the graft junction. This asymmetry is relieved after cell-to-cell transport across the forming junction resumes. Cytokinin also plays a key role in graft formation, in part through its interactions with auxin. Cytokinin is primarily produced in roots and moves to the shoot through the xylem, and thus accumulates in the rootstock half of the graft. Cytokinin regulates the placement of PIN proteins in developing vasculature which, in turn, regulates the movement of auxin. However, the dynamic interaction of these two hormones, their intercellular movement, and their role in establishing the placement of new vascular connections during graft formation is not well understood. Here, I present my work using a DR5/TCS auxin/cytokinin dual reporter line in tomato to track the dynamics of these two hormones during grafting. Using this line, I am able to quantify the relative response of auxin and cytokinin above and below the graft junction within a pivotal developmental window from 2-4 days post-grafting. This work provides a useful framework for understanding the role of auxin and cytokinin in specifying vascular reconnections during the regenerative process of graft formation.

P-05-05

Towards a better understanding of auxin-regulated intercellular communication during lateral root formation

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During *Arabidopsis thaliana* lateral root formation, endodermal cells progressively decrease their volume whilst maintaining membrane integrity, thereby spatially accommodating the expansion growth of the underlying lateral root primordia. This volume loss is triggered by Aux/IAA-mediated auxin signaling, with a prominent role of *IAA3/SHY2*. In the *CASP1pro::shy2-2* mutant, a stabilized form of SHY2 is expressed in differentiated endodermal cells. As a result, auxin-mediated transcriptional responses that are required for pericycle – endodermis communication are blocked, resulting in complete inhibition of lateral root formation. By characterizing the responses of *CASP1pro::shy2-2* to exogenous auxin treatments, we addressed the capacity of the native auxin, IAA, and two synthetic auxins, NAA and 2,4-D, to restore lateral root formation in *CASP1pro::shy2-2*. Specifically, IAA and NAA treatment can partially rescue lateral root phenotype in *CASP1pro::shy2-2*, whereas 2,4-D treatment cannot. Unlike IAA and NAA, 2,4-D induces uncontrolled cell division without proper morphology of lateral root primordia. In addition, we mapped the transcriptional outputs of auxin and cytokinin in responses to exogenous auxin treatments across *CASP1$_{pro}$::shy2-2* primary root to investigate how their signalling pathways interact to override the absence of endodermal spatial accommodation. Our results provide important insights into how auxin and cytokinin signalling are affected in this mutant. This in turn could enhance our understanding of auxin-mediated spatial accommodation responses during lateral root development in *Arabidopsis*.

P-05-06*

New insights on evolution of auxin signaling in early green plants

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Auxin is an essential hormone for plant growth and development. However, when and how during evolution auxin established this prominent role remains a mystery. It is assumed that auxin as a signal molecule originated during the transition of plants from water to land. This assumption is based on the fact that the canonical auxin signaling pathway is absent in green algae – the aquatic ancestors of land plants. Nevertheless, we discovered a species of the chlorophyte green alga *Draparnaldia* that has astonishing morphological complexity and responds to auxin. This discovery shows that studies in algae may provide completely novel insights into alternative molecular mechanisms of plant auxin responses. Until now auxin responses (i.e. metabolomics, transport, signaling) have been systematically studied only in land plants, not in algae. To close this knowledge gap, we study auxin responses in two green algae: *Draparnaldia* (a chlorophyte alga that we develop as a new model) and *Klebsormidum* (an emerging streptophyte model). Here: (1) I will introduce *Draparnaldia* as a new algal model system. (2) I will present our first insights into auxin responses in *Draparnaldia* and *Klebsormidium*, comparing them to the auxin responses known from land plant *Arabidopsis*. Overall, our results strongly suggest that auxin in algae functions as a signal molecule. They also highlight the importance of establishing the new chlorophyte algal model, *Draparnaldia*, to better understand (i) the alternative evolution of morphological complexity in plants and (ii) the original roles of auxin in green plants before the conquest of land.

P-05-07

Hormonal signaling in shoot apical meristem in *Physcomitrium patens* revealed by single nuclei RNA-seq

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The shoot apical meristem (SAM), containing multipotent stem cells, is the ultimate source of shoot structure in land plants. However, how the mechanisms specifying stem cell identity in the SAM evolved is not well understood. The SAM of bryophytes contains a single stem cell, called an "apical cell", in contrast to the seed plants SAM which contains multiple stem cells. The SAM with a single apical cell is commonly observed in bryophytes and most ferns, suggesting that the SAM with a single stem cell is the original type. To obtain insight into the mechanisms specifying apical cell identity in bryophytes, we conducted single-nuclei RNA-seq analysis on *Physcomitrium patens* (*P. patens*). The apical cell reiterates asymmetric cell divisions, producing daughter cells to be maintained as a stem cell and to differentiate. Cell clustering and trajectory analysis revealed dynamic changes in gene expression patterns during the apical cell specification and cell differentiation from the apical cell. Notably, genes involved in cytokinin metabolism are upregulated in the cell cluster containing the apical cell. On the other hand, genes related to auxin signaling are upregulated during leaf and stem differentiation. It is well known that cytokinin levels are high in the stem cell region, and auxin levels are high in differentiating cells in seed plants. In *P. patens*, cytokinin promotes apical cell formation. Therefore, our results indicate that the hormonal signaling distribution is conserved in the SAM of land plants. We propose that cytokinin acts as a conserved stem cell factor in land plants.

P-05-08

Cytokinin modulation of the microtubular cytoskeleton

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The primary roles of auxin and cytokinin are well established, however, most investigations into the molecular mechanisms underlying the cytokinin hormonal pathway focus on the auxin- cytokinin crosstalk, leaving much to be uncovered about the other cytokinin-regulated pathways and their targets. One such novel target of cytokinin is the microtubular cytoskeleton. In rapidly elongating epidermal cells of the root, cytokinin re-orients the cortical microtubule array and stabilizes the ends of individual microtubules. Investigation reveals the CRE1/AKH4 receptor and the transcription factor ARR1 as upstream components of this pathway. No further downstream components have been identified so far. We describe a multi-level approach to further characterize this effect of cytokinin on microtubules: first, the role of microtubule associated protein candidates differentially phosphorylated after cytokinin treatment; second, points of interference in the mechanism of microtubule array re-orientation; and finally, the effect of cytokinin on the individual microtubule end dynamics.

P-05-09

Investigating the role of CYCLIN-P3s in Arabidopsis style development

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The establishment of a symmetry type, *i.e.*, radial and bilateral symmetry, during organogenesis is a fundamental process shared among multicellular organisms. The *Arabidopsis* gynoecium, which subsequently forms the fruit, is an ideal system to explore how master transcriptional regulators balance the activity of phytohormones to control the bilateral-to-radial symmetry transition at gynoecium apex.

The transcription factor SPATULA (SPT) promotes differentiation of the apical style by sustaining auxin distribution, on one hand, and repressing the cell-division input promoted by the hormone cytokinin, on the other hand.

We found two members of the P-type family of cyclins (CYCLIN-Ps), named CYCP3;1 and CYCP3;2 (hereafter CYCP3s), negatively regulated by SPT, and positively regulated by the phytohormone cytokinin (CK). Furthermore, our work shows that CK and SPT activity in regulating *CYCP3s* expression is independent and antagonistic to one another. Consistently, CYCP3s are partially sufficient and necessary to break radial style symmetry at the gynoecium apex, as overexpression of *CYCP3s* leads to a break in radial style symmetry and the triple mutant of *spt cycp3;1 cycp3;2* partially rescues the bilateral-style phenotype of *spt* single mutant. Additionally, increasing cell-division rate (by exogenous CK-treatment) in mutants of cell-division orientation, i.e., *trm678*, *ton1a* and *pok1*,2 leads to split-style phenotypes, mimicking the *spt* phenotype. Altogether, our data corroborate the hypothesis that control of cell-division rate and orientation, particularly in the apical-medial cells of gynoecium, is orchestrated by SPT via fine-tuning the auxin/cytokinin balance and it is fundamental to guarantee correct anisotropic tissue-growth and form a radially symmetric style.

P-05-10*

Post-translational modification of SPATULA by SECRET AGENT and SPINDLY promotes organ symmetry transition at the gynoecium apex

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The establishment of organ symmetry during multicellular development is a fundamental process shared by most living organisms. Here, we investigated how two *O*-glycosyltransferase enzymes of *Arabidopsis thaliana*, SPINDLY (SPY) and SECRET AGENT (SEC) synergistically promote a rare bilateral-to-radial symmetry transition during patterning of the plant reproductive organ, the gynoecium. SPY and SEC modify N-terminal residues of the bHLH transcription factor SPATULA (SPT) *in vivo* and *in vitro* by attaching *O*-fucose and *O*-linked-β-N-Acetylglucosamine (*O*-GlcNAc), respectively, to promote style development. This post-translational regulation does not impact SPT homo- and hetero-dimerisation events with INDEHISCENT (IND) and HECATE 1 (HEC1), although it enhances the affinity of SPT for the kinase *PINOID* (*PID*) gene locus to promote transcriptional repression. Our findings reveal a previously unrecognized mechanism for *O*-GlcNAc and *O*-fucose post-translational decorations in controlling style development and offer the first molecular example of a synergistic role for SEC and SPY in plant post-embryonic organ patterning.

P-05-11

AtELP4, a subunit of Arabidopsis Elongator complex, regulates cell proliferation, curling, and dorsoventral polarity in leaf morphogenesis

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The Elongator complex plays crucial roles in eukaryotic organisms, contributing to various physiological processes, including transcriptional control, DNA replication and repair, and chromatin accessibility, by conserving tRNA modification functions. The complex consists of six subunits, with ELP1-3 forming the core subcomplex and ELP4-ELP6 the accessory subcomplex. In Arabidopsis, the Elongator complex is also conserved. This study aimed to analyze the binding of Arabidopsis subunits and compare the accessory subcomplex structure between yeast and Arabidopsis. Additionally, the research explored the plant-specific functions of AtELP4, a subunit of the accessory subcomplex ELP4-ELP6, including leaf morphogenesis and evolutionarily conserved functions between yeast and Arabidopsis. The research revealed that the function of ELP4 is partially conserved in Arabidopsis and yeast, as it affects growth sensitivity towards caffeine and elevated cultivation temperature. Genetic analysis of mutants demonstrated that the AtELP4 subunit mediated cell proliferation, curling, and adaxial-abaxial polarity of leaves, and might epistatically act on DRL1 during leaf development. Overall, the findings suggest that AtELP4, as part of the plant Elongator complex, may act upstream of a regulatory pathway for adaxial-abaxial polarity and cell proliferation in leaf development. The role of auxin in regulating the Elongator complex during leaf development will also be discussed.

P-05-12*

Ocimum kilimandscharicum 4-Coumarate CoA ligase 11 negatively regulates root formation by inhibiting polar auxin transport via flavonoids accumulation

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4-Coumarate-CoA Ligase (4CL) is an important enzyme in the phenylpropanoid biosynthesis pathway. Multiple 4CLs are identified in *Ocimum* species; however, their functions remain enigmatic. In this study, we independently overexpressed three *Ok4CL* isoforms from *Ocimum kilimandscharicum* (*Ok4CL7*, *-11* and *-15*) in *Nicotiana benthamiana, Solanum tuberosum* and *Arabidopsis thaliana* for functional characterization. Interestingly, *Ok4CL11* overexpression caused a rootless or reduced root growth phenotype, whereas overexpression of other two isoforms (*Ok4CL7* and *-15*) resulted in normal adventitious root (AR) growth. Overexpression of *Ok4CL11* caused abundance of flavonoid-glycosides (i.e. kaempferol-3,7-O-bis-alpha-L-rhamnoside [K3,7R], quercetin-3-O-rutinoside), reduced auxin levels, and disrupted auxin transport and signaling, which cumulatively results in a rootless phenotype. Further, silencing of *Ok4CL11* in the OE background stimulated AR formation. Reduced auxin levels and increased K3,7 R in the middle and basal stem sections imply the disturbance of polar auxin transport (PAT) in *Ok4CL11-OE* lines. Further, *in vitro* assessment of shoot-apex and single-node experiment demonstrates decreased rates of AR formation correlating with reduced PAT. Grafting between WT and *Ok4CL11-OE* lines partially rescued the rootless phenotype. Hetero-grafts of *Ok4CL11-OE* lines (scion) and WT (root-stock) exhibit reduced AR formation, suggesting a disruption of PAT in the root-stock possibly due to enhanced accumulation of K3,7R. *In silico* studies show that auxin transporters (PINs/LAXs) have higher binding affinity to K3,7R than to auxins, indicating a potential disruption of PAT by the flavonoid-glycosides accumulated in *Ok4CL11-OE* lines. Our study reveals a unique indirect function of Ok4CL11 as a negative regulator of root growth, likely involving inhibition of auxin transport.

P-05-13*

CHARActerize: a study of auxin efflux carriers from *Chara braunii*

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The directional transport of auxin, mediated by PIN efflux carries has been extensively studied in land plants. However, their role and function in streptophyte algae remain poorly understood. Here we present our newest results on the characterization of three auxin efflux carriers in multicellular freshwater alga *Chara braunii*. We assigned them names CbPINa, b, and c. A heterologous expression in tobacco BY-2 cells was used to study their intracellular localization, complemented with radioactively labeled auxin transport assays. GFP-tagged Chara PINs show localization on the endoplasmic reticulum and plasma membrane, with the activity in the transport of auxin. Furthermore, we performed an immunolocalization study of CbPINa and CbPINc in several *Chara braunii* cell types and developmental stages. Finally, an effect of auxin was followed in germinating oospores using finely tuned time-lapse imaging on a vertical spinning disc microscope. Overall, we demonstrate that Chara PINs with a long hydrophilic loop and localization to the plasma membrane are able to transport auxin. These findings provide insight into a conserved function of PIN auxin efflux carriers between land plants and streptophyte algae.

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P-05-14

Cytokinin oxidase/dehydrogenase 2 of barley as a target for yield improvement

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Cytokinin is degraded by cytokinin oxidase/dehydrogenase (CKX) enzymes. Mutation of *CKX* genes causes increased cytokinin concentrations in the tissues where they are expressed inducing local changes of growth and development. It has been shown that certain *CKX* gene mutations causing elevated cytokinin concentrations in reproductive tissues of monocot (rice) and dicot (oilseed rape) crop plants increase seed production and thus yield. In rice, the reduced activity of one member of the CKX protein family, OsCKX2, was shown to enhance grain number. In wheat, different yield-related traits including grain size and number were found to be associated with OsCKX2 orthologs. These results motivated us to investigate whether two orthologous *HvCKX2* genes of barley, which is another important cereal belonging to the Poacea, play a role in regulating seed yield. Both orthologous genes, *HvCKX2.1* and *HvCKX2.2*, are expressed in reproductive tissues with peak expression during the early stages of seed development. Single mutants and the *Hvckx2.1 Hvckx2.2* double mutant were generated in the barley cultivar Golden Promise using CRISPR/Cas9. We will report first results of phenotypic analysis of these mutants with a focus on yield-related traits.

P-05-15

The role of TGW6 in the determination of grain size in barley

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The *TGW6* (*Thousand Grain Weight* 6) gene that encodes indole-3-acetic acid (IAA) glucose hydrolase had been shown to be significantly associated with grain yield in rice and wheat. Non-functional TGW6 has a pleiotropic positive effect on grain weight and size, particularly in rice. TGW6 acts directly by regulating IAA supply and controlling the transition from syncytial into the cellular stage of grain development, thus controlling the number of endosperm cells and finally the size of grains. Moreover, TGW6 also acts indirectly in leaf sheaths by regulating gene expression controlling the carbohydrate storage capacity and translocation to developing grains. Apparently, TGW6 seems to be a promising target for the improvement of grain yield in cereals. Using the rice sequence, we have identified several putative *TGW6* homologs in the barley genome. We have focused on the homolog previously shown to be associated with grain size in the genome-wide association study and analyzed its expression in different stages of development of inflorescence by RT-qPCR. Screening the genetic diversity on 100 accessions of spring barley landraces, we have determined 2 variants with different protein compositions, affecting the predicted 2D structure. Interestingly, we have found a correlation between the 2D structure and the width of grains. Finally, we used CRISPR-Cas9 methodology to knock-out *HvTGW6* in the spring barley reference Golden Promise. We expect that the absence of the gene will affect the size of grains.

P-05-16

The role of auxins and cytokinins in determining root-sprouting ability of plants

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Plant communities in nature are often damaged or destroyed by various natural factors and human activities. To survive such disturbances, plants are able to regenerate by resprouting from bud banks. After damage, most plants resprout from axillary buds on stems. However, some species use buds that form adventitiously on roots; this ability is called root sprouting (RS). Thus, RS provides an evolutionarily independent alternative to axillary stem branching and an efficient survival strategy against adverse conditions. Despite the obvious advantage of effectively protecting roots deep in the soil, only about 10 % of temperate European flora exhibit the RS ability.

Knowledge about possible physiological constraints of the RS ability is still very limited. Phytohormones certainly play an important role, but it is not yet known how RS and non-RS plants differ in terms of phytohormone profiles, contents and ratios. Using congeneric pairs of RS and non-RS species grown in greenhouse experiments, and a sophisticated LC-MS methodology, we investigated whether (and how) phytohormones might be responsible for the presence of RS in plants.

Our study revealed that intrinsic phytohormone regulation, particularly the ratio of auxins to cytokinins, could contribute significantly to RS ability. All RS species were found to have significantly lower auxin/cytokinin ratios than non-RS species. In addition, RS species were generally found to produce higher levels of gibberellins than RS species. Our results contribute significantly to the understanding of the relevant hormonal factors for bud bank formation on roots rather than stems and will be discussed at the conference.

P-05-17

LAZY1 pathway induces polarity in silver birch (*Betula pendula*) branches

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LAZY1 is a key protein that contributes to branch and tiller angle in herbaceous and woody species such as Arabidopsis, rice and peach. LAZY1 has been studied for over a decade but its molecular function remains vague. However, it is known that LAZY1 participates in polar auxin transport. We have previously learnt that Young´s weeping birch has a premature stop codon in *LAZY1* gene. We have created *LAZY1* RNAi lines that phenocopy the weeping phenotype. In addition, we have created pLAZY1-erVen lines to confirm that *LAZY1* is expressed in the shoot endodermis in silver birch. To analyse auxin flow, we are currently propagating pPIN3-erVen and DR5v2-erVen lines in WT and *lazy1* background. We have conducted bulk RNA-seq on the upper vs lower parts of branch tips in the BC1 segregating population. This analysis provided a handful of genes that display LAZY1 mediated polar expression in the branch tips. These genes include known and novel putative transcriptional regulators that are possibly involved in polar growth of branches. We have also conducted X-ray diffraction and mechanical strength assay in WT vs *lazy1* branches. Our preliminary results indicate that the orientation of cellulose microfibrils might be narrower and material strength stronger in the upper part of WT branches compared to the *lazy1* mutant. We have also learnt that LAZY1 participates in the physical positioning of the primary meristem. Our experiments demonstrate that *lazy1* mutant is not able to recapitulate reorientation of the uppermost apical meristem after the primary apical meristem is lost.

P-05-18*

Evolution of the cytokinin regulatory network in conjugating green algae: first insights from *Spirogyra pratensis*

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Zygnematophyceae (conjugating green algae) are the closest known relatives to all embryophytes, rendering them an important group for evolutionary studies related to the adaptation to terrestrial life. In the green plant lineage it is so far unknown in how far a cytokinin regulatory network contributed to the evolution of land plants. For Zygnematophyceae there is so far limited understanding of the evolutionary trajectory, and adaptations of the cytokinin system. The filamentous alga *Spirogyra pratensis* [Transeau] was proposed as a model organism for this class to investigate cytokinin metabolism and signaling. A cytokinin feeding experiment including HPLC analysis was conducted to check for the existence of a cytokinin catabolic pathway. The results showed no cytokinin depletion, which is consistent with the absence of cytokinin oxidase/dehydrogenase genes in the transcriptome of *S. pratensis*. Two orthologs of LOG genes, SpLOG1 and SpLOG2, were identified and characterized through bioinformatic analysis. Their putative function of cytokinin activation still is to be confirmed. We further screened the *S. pratensis* transcriptome and identified three putative cytokinin receptors, the cDNAs of which were cloned for characterization. In order to prepare reverse genetic analyses of cytokinin related genes, we utilized protoplast transformation, but so far only transient transformation events were obtained. In our project it is expected that deepened insights into the functionality of the cytokinin regulatory network in *S. pratensis* will be provided, which may help to understand how cytokinins contributed to the establishment of terrestrial life in the last common ancestors of Zygnematophyceae and land plants.

P-05-19*

MONOPTEROS isoform MP11ir role during somatic embryogenesis in *Arabidopsis thaliana*

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Auxin is a crucial regulator of plant morphogenesis, including during embryo development. Exogenous auxin application is necessary to induce an embryogenic response in *in vitro* cultured explants of Arabidopsis and many other plant species. Therefore, components involved in auxin transport, signaling, and metabolism are crucial for embryo formation. Those components are pivotal during embryo regeneration. And one of the transcription regulators involved in nuclear auxin signaling, AUXIN RESPONSE FACTOR 5/MONOPTEROS (ARF5/MP), appears to play a predominant function. *MP* is highly expressed during somatic embryogenesis, and its mutant is impaired in the embryogenic response. Hence, the role of MP in embryo formation is unquestioned. Our study examined the presence and importance of the MP isoform, MP11ir, during the embryogenic transition. *MP11ir* transcript is an alternative spliced variant of *MP* characterized by intron retention, translated into a truncated protein missing the PB1 domain required for dimerization. Thus, the produced MP11ir isoform is insensitive to Aux/IAA repression and could act in an auxin-independent manner. We identified a high level of *MP11ir* transcript during auxin-(in)dependent induction of somatic embryogenesis. We showed that the MP11ir isoform could partially rescue the capacity for embryo regeneration in *mpS319*. However, the truncated MP protein (ΔARF5) overexpression leads to somatic embryogenesis inhibition, and explants produce callus rather than somatic embryos. The presence of ΔARF5 modifies the expression of genes involved in auxin biosynthesis. Consequently, it alters endogenous local and global auxin levels, negatively impacting embryogenic transition.

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P-05-20

Investigating the role of auxin in patterning the petal of *Hibiscus trionum*

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Patterns on petals are not only attractive to our eyes, but can fulfill a plethora of functions, such as shielding pollen from high temperatures and UV-radiations, and attracting pollinators, hence ensuring flower fertilization and fructification. Differences in cell characteristics generates those patterns but how distinct cell fates across the petal epidermis are specified at the molecular level remain elusive. Flowers of *Hibiscus trionum*, a novel model system established to study petal patterning, show a "bullseye" pattern emerging from the contrasting appearance of the proximal and distal portions of the petals, with a bullseye boundary in between.

Since hormone signaling and cross-talks have been repeatedly co-opted during evolution, and petals can be seen as modified leaves, we hypothesize that some of the gene regulatory networks and signaling events controlling petal patterning may resemble those that establish proximo-distal polarity and developmental boundaries in leaves.

Through a petal transcriptomic approach, we identified homologs of leaf development regulators alongside factors related to auxin and cytokinin signaling and homeostasis. Then, we generated a collection of knock-out, reporter and overexpressor Hibiscus lines to characterize auxin and cytokinin response dynamics at the cellular resolution as petal develop and investigate the role of candidate genes in specifying cell fates across the petal epidermis. I will present the results that we obtained and how those will help us to progress towards our long-term goal of elucidating how co-opting and altering developmental programs allows evolution to generate the diversity of "endless forms most beautiful and most wonderful".

P-05-21*

Revealing the origins of plant hormones: A profiling perspective

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The molecular background of most phytohormone responses is not conserved between land plants and closely related streptophyte algae. However, scattered reports of endogenous phytohormone production in these organisms exist. We performed a detailed LC/MS-based analysis primarily of auxins and cytokinins, but also ABA, jasmonates and certain phenolics in all lineages of streptophyte algae. We also included chlorophyte algae and early diverging land plants as outgroups. Free auxin, tRNA-derived cytokinins and certain phenolics including salicylic acid were found ubiquitously. However, land plants differed from green algae by the consistent detection of abscisic acid and the presence of auxin and cytokinin conjugates and trans-zeatin, supporting the hypotheses that these three phytohormones likely came to regulate development in the ancestral land plant. By contrast, we observed a patchy distribution of jasmonates among streptophytes. The culture media and control media were likewise analyzed to account for phytohormone excretion and environmental contamination, adding an unprecedented robustness to the analysis. Extracellular auxins and cytokinins were frequently detected, while agar constituted a major external source of phenolic compounds.

P-05-22

CYTOKININ OXIDASE/DEHYDROGENASE (CKX) genes of Arabidopsis and oilseed rape as targets for yield improvement

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Yield of crop plants is a complex trait controlled by numerous genes. It has been shown that mutation of certain *CYTOKININ OXIDASE/DEHYDROGENASE* (*CKX*) genes, encoding enzymes which degrade the hormone cytokinin, result in an increased cytokinin level in reproductive meristems which alter yield-related traits such as the number of flowers and seed-bearing structures in monocot (rice) and dicot (oilseed rape) crop plants. This work highlighted the relevance to increase the sink capacity of plants to achieve yield enhancement. In the allotetraploid oilseed rape combined mutations of the four *BnCKX3* and the two *BnCKX5* genes, identified by TILLING, altered the size and activity of the inflorescence meristem responsible for flower formation and the activity of the placenta of gynoecia responsible for ovule formation. These *ckx3,5* mutants developed more flowers on the main stem which contained gynoecia forming more ovules and developed more pods resulting in an increased total seed weight (Schwarz et al., J. Exp. Bot. 71, 7146-7159, 2020). However, these six *CKX* genes which are expressed during reproductive development in distinct phases and tissues may play individual roles for the different aspects of the yield phenotype, which remained unclear. To study the eventual subfunctionalization of the *CKX3* and *CKX5* genes we identified 64 different *CKX3* and *CKX5* mutant genotype combinations and will present first results on their meristem activities and yield. In Arabidopsis, we have explored the eventual role of additional *CKX* genes in regulating seed yield and identified *CKX4* as a novel yield gene acting in combination with *CKX3*.

P-05-23

The “groundbreaking” ability of the root: how to cope with soil’s hardness and avoid obstacles

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Mechanical-robotical design takes inspiration from nature since a long time. The root is the organ that supports plant’s growth by allowing its anchorage to the substrate. One of the first tasks that the root must complete is to penetrate the soil as quickly as possible to prevent the plant from being wiped out by environmental agents. Once it is in the ground, the root must avoid obstacles and face the substrate's physical properties in ever-changing environment. Biophysical models of root growth are currently available only for monocotyledons with particularly thick roots. Nonetheless, the biological mechanisms underlying the regulation of root growth into substrates with different hardness remain largely unknown. While exploiting the root of the model organism Arabidopsis thaliana, my research aims to: 1. characterize root architecture adaptation to ground-derived mechanical stresses (e.g. growth in homogeneous substrates with increasing compactness). 2. elucidate the molecular mechanisms permitting root growth in compact homogeneous substrates. Utilizing a combination of physiological, genetic, molecular biology and bioinformatics approaches, here we show that variation in auxin distribution drives the morphological changes that allow the root to cope with substrates characterized by diverse physical properties.

P-05-24

The role of auxin and cytokinin in the synergistic interaction of histone acetyltransferase GCN5 and receptor CLV1 in inflorescence and floral meristem

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GCN5 histone acetyltransferase and CLV1 receptor have been shown to interact genetically to affect gynoecium development. In *clv1-1gcn5-1* double mutants, gynoecia have reduced ovary and enlarged stigmatic and stylar regions. A transcriptomic approach was utilised to elucidate the molecular and hormonal aspects of GCN5 and CLV1 synergy. RNA-seq was performed in wild-type, *gcn5-1*, *clv1-1* and *clv1-1gcn5-1* double mutants from inflorescence tissue, including flowers up to stage 13. A total of 27054 genes were expressed in the inflorescences across all genotypes. Analysis has shown that 24.42% of the total genes were differentially expressed (DEG) in *gcn5-1*, 13.82% in *clv1-1* and 36.0% in *clv1-1gcn5-1*. In auxin homeostasis, both biosynthesis and transport were impaired in *clv1-1gcn5-1.* Genes involved in auxin biosynthesis and the PIN family of auxin efflux transporters were among the downregulated genes. Most of the DEG in these families were common in *gcn5-1* and *clv1-1gcn5-1*, suggesting that this was primarily a *gcn5*-dependent effect. In cytokinin responses, *clv1-1gcn5-1* exhibited a reduction in cytokinin biosynthesis since many genes, like members of the LONELY GUY family, were down-regulated. Similarly to auxin homeostasis, this effect is also mostly Gcn5 dependent. The downregulation of cytokinin response genes of the CRF family was also observed in the double mutants. These transcriptomic data support the hypothesis that *GCN5* and *CLV1* synergistically affect inflorescence and floral meristem development by modulating auxin and cytokinin homeostasis and signalling.

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P-05-25

Single cell analysis of the CLE45-BAM3 pathway in root development

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The plant vasculature delivers phloem sap to the growth apices of sink organs, namely the meristems, via the interconnected sieve elements of the protophloem. In the root meristem of Arabidopsis thaliana, stem cells give rise to two files of protophloem sieve elements (PPSEs), whose timely differentiation relies on a set of positive genetic regulators.

CLE peptides constitute a crucial class of plant signal peptides that have been implicated in plant development, stress response, and growth regulation. Prior research has demonstrated that CLE45 is capable of impeding protophloem formation, and we have recently identified the receptor kinase BAM3 as the specific receptor of CLE45, following a series of experiments. In numerous mutants with defective protophloem development, blocking the CLE45-BAM3 pathway has been shown to partially restore the deficits of protophloem and root development. These findings collectively suggest that the CLE45-BAM3 pathway plays a pivotal and distinctive role in protophloem development.

Currently, the downstream effects of the CLE45-BAM3 pathway remain elusive. To unravel the molecular details of this pathway, we performed time-course CLE45 treatments on plants, followed by analysis of the downstream gene response of CLE45-BAM3 pathway using single-nucleus sequencing technology. Based on the resulting transcriptomic data, the molecular mechanism regulating phloem development by CLE45 will be more comprehensively elucidated.

P-05-26

Plant hormone and peptide signaling converge in the regulation of cambium activation in Arabidopsis roots

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Plant secondary growth is driven by two concentric meristems, the inner vascular cambium and outer cork cambium. The cambial periclinal cell divisions of both meristems, providing thickness to plant organs, are activated soon after the primary development. Cytokinins and a set of downstream transcription factors are key players in promoting transition from primary to secondary development, however it is unknown whether other factors play a role in this transition. Here, we show that in addition to cytokinins, also auxin and TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) peptide signaling are regulating the timely activation of secondary growth in Arabidopsis roots. Time-course transcriptomic analysis after cytokinin-treated, cytokinin deficient *isopentenyltransferase1,3,5,7* (*ipt1,3,5,7*) mutant revealed upregulation of genes encoding the TDIF peptide as well as its receptor PHLOEM INTERCALATED WITH XYLEM (PXY). Cytokinin treatment upregulated also plant hormone auxin signaling, and concomitantly auxin and TDIF signaling were found to be required for proper cytokinin response. Furthermore, auxin and cytokinin signaling acted synergistically in promoting secondary development. Bioinformatic analysis suggested that transcription factors belonging to the DNA-BINDING WITH ONE FINGER (DOF) and ETHYLENE RESPONSE FACTOR (ERF) gene families may be regulated by cytokinins, auxin and TDIF which led us to characterize these candidate genes further. Overall, we find that cytokinins, auxin and TDIF form a tightly intertwined network of positive regulators for activation of secondary growth in the Arabidopsis root.

P-05-27

Arabidopsis lateral shoots display two distinct phases of growth angle control

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Lateral shoot branches are fundamental elements shaping plant architecture above-ground. In Arabidopsis, the formation and activation of axillary meristems, from which shoot branches originate, are driven by a complex network of factors, with auxin and cytokinins playing a major role. Once they have grown out from the main stem, lateral shoots set and maintain non-vertical growth angles with respect to gravity, also known as gravitropic setpoint angles (GSAs). GSA regulation is based on two counteracting auxin-dependent mechanisms: the antigravitropic offset and gravitropic response. We have identified an early phase of shoot branch development that precedes GSA maintenance. During this phase, despite being competent to respond gravitropically, the growth angle development of the young branch is independent of gravity and is an important component of final growth form of the branch. Here, we describe the biophysical and molecular basis of this early pre-GSA stage of branch development, including transcriptomic analysis comparing adaxial and abaxial sides of young shoot branches. Understanding this early phase of growth angle development in shoot branches will allow a more complete model shoot architectural control, potentially identifying new targets for the manipulation of canopy architecture in crop species.

Interactions and Cross-talk

P-06-01*

Crucial role of asymmetric cell division in plant adaptation to hard and dry soils

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The global climate change is progressively hardening soils due to the progressive advancement of dry-lands. Recent studies suggest that root cortex tissue is fundamental for roots ability to penetrate hard and dry soils. Plants show a high interspecific variability regarding the number of cortical layers, spanning from one, as in the Arabidopsis thaliana model species, to several as in horseradish. However whether the variability of this trait is correlated with penetration of soils possessing different hardness remain unknown. Utilizing Cardamine, an Arabidopsis close relative showing 2 cortical layers, we showed that cortical layer number variability is under the control of a complex molecular mechanism, among which cytokinins (CK) possess a major role. The goal of my PhD project is to understand whether an increase in cortical layer number might help plants to penetrate soil of increasing hardness. To this end I modulated cortical layer number in Arabidopsis and Cardamine plants varying the levels of CK in a tissue-specific manner. Then, I tested how penetrative capacity of the obtained mutants varies in media showing incremental hardness. My data enlighten for the first time how modulation of cortical layer number correlate with improving root penetrative capacity in hard soils.

P-06-02

Endogenous level of brassinosteroids is positively regulated by auxin signaling in *Arabidopsis thaliana*

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Brassinosteroids (BRs) and auxin frequently show similar regulatory activities in growth and development of plants. To understand how both hormones are positively interactive in growth and development of plants, homeostatic regulation of endogenous BRs by auxin signaling was investigated in Arabidopsis plant. Abnormal growth of *arf7* and *arf7 x arf19* where auxin transcription factor ARF7 and ARF19 are deficient was partly restored to growth of wild type by exogenously applied BRs. In *arf7* and *arf7 x arf19*, expression of *CYP85A1* as a BR biosynthetic genes was down-regulated, while expression of *BAS1*, a catabolic gene was up-regulated compared to those in wild type. By the altered expression of the genes, the endogenous level of a bioactive BR, castasterone, was greatly decreased in *arf7* and *arf7 x arf19*. EMSA and ChIP analysis demonstrated that ARF7 binds to auxin regulatory elements present in the promotor region of *CYP85A1* and *BAS1*, suggesting that ARF7 directly regulates expression of both biosynthetic and catabolic genes to control homeostasis of endogenous BRs in Arabidopsis. Yeast two hybrid assay showed ARF7 directly interacts with BZR1, which inhibits their DNA binding activities to regulate expression of *CYP85A1* and *BAS1* in the plant. Coupled with binding of BZR1 to the promotor of *CYP85A1* and *BAS1*, this suggests that ARF7 and BZR1 mutually compete for binding to the *CYP85A1* and *BAS1* promoter to regulate endogenous level of BRs in Arabidopsis.

P-06-03*

Gibberellins promote polar auxin transport to regulate stem cell fate decisions in cambium

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Plant secondary growth is conducted by a lateral meristem called vascular cambium. Vascular cambium contains bifacial stem cells, which produce secondary xylem inwards and secondary phloem outwards. However, it is unknown how these fate decisions are regulated. Here we show that the positioning of an auxin signalling maximum within the cambium determines the fate of stem cell daughters. The position is modulated by gibberellin-regulated, PIN1-dependent polar auxin transport. Gibberellin treatment broadens auxin maximum from the xylem side of the cambium towards the phloem. As a result, xylem-side stem cell daughter preferentially differentiates into xylem, while phloem-side daughter retains stem cell identity. Occasionally, this broadening leads to direct specification of both daughters as xylem, and consequently, adjacent phloem-identity cell reverts to being stem cell. Conversely, reduced gibberellin levels favour specification of phloem-side stem cell daughter as phloem and the xylem-side daughter keep their stem cell identity. Together, our data provide a mechanism by which gibberellin regulates the ratio of xylem and phloem production.

P-06-04

Phosphorylation of AHP3 by phyA – a crosstalk mechanism between light signaling and MSP?

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For decades, the light is known to interfere with cytokinin-mediated regulations. Although numerous mechanisms underlying the interaction have been described, our knowledge on the light-dependent regulations of other signaling pathways remains fragmental. Here, we demonstrate the ability of the far-red light photoreceptor phytochrome A (phyA) to phosphorylate the *Arabidopsis* His-containing phosphotransfer protein AHP3, acting as a signal transmitter in the multistep phosphorelay (MSP) pathway.

We developed a panel of antibodies recognizing AHP proteins. Using antibodies recognizing the dephosphorylated form of AHP3, we detected phosphorylation of endogenous AHP3 in protein extracts obtained from suspension cultures in *Arabidopsis WT*, ecotype *Columbia-0*. In contrast, the AHP3 phosphorylation was not observed in phyA loss-of-function mutant grown under different light conditions (dark, white, red, and far-red). The phyA was expressed and purified in *E. coli* and shown to possess autokinase activity. The ability of phyA to phosphorylate AHP3 was confirmed by *in vitro* kinase assay. Using MS analysis, we found that phyA phosphorylates AHP3 *in planta* on one of the four Ser residues (Ser87-Ser90) adjacent to conserved phosphoaccepting His82 in the light-independent way. Our findings also suggest that a fusion of AHP3 with GFP is unsuitable for the immunodetection of the AHP3 phosphorylation.

Altogether, our results suggest a novel molecular mechanism of light integration into plant hormonal signaling via phyA-mediated AHP3 phosphorylation.

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P-06-05

Energy homeostasis dependent modulation of phytohormone metabolism upstream of root growth control

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Loss of the catalytic subunit (KIN10) of the cellular signalling hub SUCROSE NON-FERMENTING RELATED KINASE 1 (SnRK1), an evolutionarily conserved kinase complex involved in cell morphology and metabolome regulation, results in reduced plant fitness (Nukarinen et al., 2016; Retzer and Weckwerth, 2021). Furthermore, plants evolved a network of intracellular processes to adapt to environmental changes that depend on fine-tuned modulation of phytohormone abundance, distribution and metabolism (Retzer and Weckwerth, 2023). Our results show that SnRK1 activity is required to orchestrate phytohormone homeostasis in roots, which is changing with altered growth conditions. We are investigating how changing levels of KIN10 activity results in pronounced changes in root trait establishment, including root hair outgrowth, lateral root formation and directional root growth control due to misregulated phytohormone homeostasis.

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P-06-06

A Role for Auxin-Cytokinin Synergism in Nutrient Starvation and Plant-Fungal Mutualism

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Plants are capable of developing new organs and regulating their growth throughout their lifespan. Phytohormones, particularly auxin and cytokinin, play crucial roles in these processes. The Benkova lab recently discovered the SYNERGISTIC ON AUXIN AND CYTOKININ 1 (SYAC1) gene in Arabidopsis thaliana whose expression is strictly dependent on simultaneous activity of auxin and cytokinin signalling pathways. SYAC1 is the first gene whose expression in root tissue is known to be induced by synergistic action of both hormones. By inhibiting pectin secretion, SYAC1 renders cell walls softer and reduces elongation growth. While this functionality is important in several developmental contexts in shoot tissue, SYAC1's function in the root remained unknown at first. In setting out to unravel its function in root tissue we discovered that limited nutrient availability triggers SYAC1 expression. Plants adapt their root system architecture in response to nutrient starvation, a process requiring auxin, cytokinin and cell wall modification. SYAC1 expression increases further if a mutualistic fungal endophyte is present under nutrient starvation. Biotic interaction with soil microbes such as fungi and rhizobia involves auxin, cytokinin as well as cell wall regulation. We hypothesise that SYAC1, driven by auxin-cytokinin synergism, is involved in both, regulation of root system architecture upon nutrient starvation and in regulating interaction with soil microbes via softening the cell walls, thereby facilitating colonisation.

P-06-07

Cytokinin-deficient CRISPR/Cas9 mutants of *Chlamydomonas reinhardtii* show reduced ability to prime resistance of tobacco against bacterial infection

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Microalgae play an essential role in agriculture as bio-fertilizers and biostimulants, but some also produce various plant hormones, such as cytokinins (CK). CK are a class of natural plant hormones and there is evidence that exogenous or bacterial CK can modulate plant defense. However, there is a lack of knowledge about the effect of specific microalgae or microalgae-produced CK on this response. In our study, we have evaluated the CK-producing microalgae *Chlamydomonas reinhardtii* (*Cri*) on its ability to prime *Nicotiana tabacum* plant defenses against its natural pathogen *Pseudomonas syringae* pv. *tabaci*. Our work functionally verifies that *Cri* primes plant defense response, with cytokinins being a crucial component. We have used the CRISPR/Cas9 system to generate *Cri* LOG and IPT gene knockouts, which are major points of the CK biosynthesis pathways. To our knowledge, these are the first algae CK-KO lines created, and could serve as great tools for elucidation of CK role in algae.

While *Cri* shows strong protection potential, the CK-deficient mutants have a reduced ability to affect plant defense, where the degree of protection correlates with the CK levels – the IPT mutants show less protection than the LOG mutants. Additionally, by measuring plant defense gene expression by RTq-PCR we have shown that *Cri* treatment stimulates tobacco defense response by priming.

P-06-08

ETR1/AHK5 mediated activation of MSP – a novel mechanism of cytokinin and ethylene signal integration

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Both cytokinin and ethylene are phytohormones with great importance to plant growth and development. It has been shown the multistep phosphorelay (MSP) pathway, which mediates cytokinin signaling, can be activated by ethylene in ETR1-dependent way specifically in the root transition zone to control the root apical meristem size.

Ethylene and cytokinin sensors have common origin in the bacterial two-component system. The ethylene receptor ETR1 contains both a histidine kinase (HK) domain and a receiver domain (RD), components essential to the transduction of signal via MSP. However, while the HK domain of ETR1 is active allowing ETR1 autophosphorylation, ETR1 RD cannot accept the phosphate, thus preventing the ETR1 to directly control MSP pathway. *In vitro* kinase assay showed that after autophosphorylation on its HK domain, ETR1 is able to phosphorylate the RD of another HK, AHK5, instead. The phosphate is then transferred to AHP1/2/3, downstream components of MSP signaling, suggesting ETR1/AHK5 work together to activate the MSP in ethylene-dependent way. To study the effects of the ETR1-AHK5 mediated phosphorelay *in planta*, we designed a chimera consisting of full length ETR1 with its RD swapped for RD of AHK5. In *ahk5-1 TCSn:GFP* lines the ETR1-AHK5 chimera, controlled by the endogenous *ETR1* promoter, MSP was activated in the root transition and elongation zones, demonstrating the functionality of the ETR1/AHK5 mediated phosphorelay *in planta*. The effects of ethylene precursor ACC and/or gaseous ethylene on the ETR1-AHK5 chimera to control MSP signaling will be presented.

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P-06-09

Cytokinin regulated endomembrane trafficking to coordinate plant organogenesis

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Although solo roles of auxin and cytokinin have been extensively researched, dissecting mechanisms underlying their interaction remains challenging. Cytokinin interference with endomembrane trafficking of PIN1, resulting in lytic degradation of this auxin transporter, has been observed recently. However, the molecular mechanism of how cytokinin mediates this process remains elusive. We have undertaken in-depth analyses of posttranslational modifications to shed light on this unknown pathway, specifically focusing on phosphorylation. By performing a phosphoproteome analysis of *Arabidopsis thaliana* roots treated with cytokinin several potential candidates underlying cytokinin-regulated PIN trafficking have been identified. Functional characterization of the candidate proteins is an important step towards unlocking the novel mechanism behind the rapid fine-tuning of polar auxin transport.

P-06-10

Role of B-family GATA transcription factors in the nitrogen- and cytokinin-responses of *Physcomitrium patens* and *Arabidopsis thaliana*

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GATAs are evolutionarily conserved transcription factors that bind the consensus sequence W-G-A-T-A-R. In *Arabidopsis thaliana*, four classes of GATAs are known, and among them the class B-GATAs have been intensively studied. Nitrogen (N) and cytokinin (CK) are upstream regulators of B-GATAs, with the B-GATAs *AtGNC* (*GATA, NITRATE-INDUCIBLE, CARBON METABOLISM-INVOLVED)* and *AtGNL/AtCGA1* (*AtGNC-LIKE/CYTOKININ-RESPONSIVE, GATA1*) described as being positively regulated by application of N and CK, respectively. However, the role of B-GATAs in N- and CK-responses is still elusive and not fully understood. By using newly generated and existing subsets of *B-GATA* mutants from the model organisms *Physcomitrium patens* and *Arabidopsis thaliana,* we aim to gain a better understanding of the roles of these B-GATAs in N- and CK-dependent responses. Investigation of protonema growth induced in *P. patens* by N- or CK showed a differential response in the PpB-GATA mutants when compared to the wild type. In line with these findings from Physcomitrium, we observed a differential response in *B-GATA* Arabidopsis mutants in response to N- and CK-treatment. Our follow up experiments, such as investigations of CK-reporter gene activities in the different *B-GATA* mutant backgrounds, will lead to a better understanding of their roles in N and CK signalling in *P. patens* and *A. thaliana,* and the degree of conservation of these roles between the two model organisms.

Responses to Environmental Stimuli

P-07-02

PLASTID LIPASE2 participating in jasmonate biosynthesis is involved in regulation of Arabidopsis cold acclimation under standard and low light conditions

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To get a novel insight into light requirement for cold acclimation in Arabidopsis, we compared transcriptome reprograming at early phase of cold acclimation under standard (100 µmol.m^{-2}.s^{-1}, SPFD) and decreased (20 µmol.m^{-2}.s^{-1}, DPFD) photon flux density. Among differentially regulated genes, *PLIP2* was found to be upregulated to a significantly higher extent when cold treatment was performed under DPFD. Previously, *PLIP2* was shown to encode a plastid phospholipase A1 cleaving monogalactosyldiacylglycerol, and *PLIP2* overexpression resulted in increased levels of bioactive forms of jasmonates. Here we show that *PLIP2* belongs to key genes regulating an extent of cold acclimation under standard and decreased photon flux densities.

P-07-03

Nitrogen deficiency- and sucrose-induced anthocyanin biosynthesis is modulated by HISTONE DEACETYLASE15 in Arabidopsis

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Anthocyanin accumulation is a hallmark response to nitrogen (N) deficiency in Arabidopsis (*Arabidopsis thaliana*). Although the regulation of anthocyanin biosynthesis has been extensively studied, the roles of chromatin modification in this process are largely unknown. N deficiency-induced anthocyanin accumulation is modulated by HISTONE DEACETYLASE15 (HDA15) in Arabidopsis seedlings. The *hda15-1* T-DNA insertion mutant accumulated more anthocyanins than the wild type when the N supply was limited. Enhanced anthocyanin accumulation is caused by up-regulation of anthocyanin biosynthetic and regulatory genes in the mutant. The up-regulated genes also have increased levels of histone acetylation in the mutant. The accumulation of anthocyanins induced by sucrose and methyl jasmonate, but not that induced by H_2O_2 and phosphate starvation, was also further enhanced in the *hda15-1* mutant. While sucrose increases histone acetylation in genes in a similar manner to that caused by N deficiency, methyl jasmonate only enhances histone acetylation in genes involved in anthocyanin biosynthesis in the *hda15-1* mutant. These results suggest that different stresses act through distinct regulatory modules to activate anthocyanin biosynthesis and that HDA15-mediated histone modification modulates the expression of anthocyanin biosynthetic and regulatory genes to avoid anthocyanin over-accumulation in response to N deficiency and other stresses.

P-07-04

Cytokinins in response to a combination of high light and heat stress in rice seedlings

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Plants respond to multiple stresses of abiotic environmental factors with a high degree of complexity. Light and temperature are two primary factors which influence plant growth and development. Rice plants were treated under control conditions, high light, heat stress and the combination of the two treatments. The heat treatments were aimed at shoots only / roots only / the whole plant. The photosynthetic parameters, growth parameters, complex hormonal profiling, and selected transcriptomic profiling was evaluated under the given conditions. The main hormones that responded specifically to the combination of heat and high light were cytokinins, however, the individual cytokinins (tZ, cZ, iP, DZ) responded with different patterns. tZ was the only cytokinin that was directly influenced by the light quality probably due to its direct involvement in photosynthetic activity. These results support a theory of different cytokinins having different roles in plant growth and development.

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P-07-05

MPK-mediated phosphorylation of IAA15 inhibits lateral root development in response to drought

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Since plants are sessile organisms, developmental plasticity in response to environmental stresses is essential for their survival. Upon exposure to drought stress, the lateral root development is suppressed to induce drought tolerance. However, the molecular mechanism to explain how the development of lateral roots is inhibited by drought stress is largely unknown. Here, we identified a repressor of auxin signaling, IAA15, as a novel substrate of mitogen-activated protein kinases (MPKs). IAA15 was directly phosphorylated by both MPK3 and MPK6 at the Ser-2 and Thr-28 residues. Interestingly, transgenic plants overexpressing the phospho-mimicking mutant of IAA15 ($IAA15^{DD}$ OX) showed reduced lateral root development due to a higher accumulation of IAA15. In addition, we showed that MPK-mediated phosphorylation by mannitol treatment strongly increased the stability of IAA15 through the inhibition of polyubiquitination. Furthermore, $IAA15^{DD}$ OX plants showed the transcriptional down-regulation of two key transcription factors *LBD16* and *LBD29*, responsible for lateral root development. Overall, our study provides the molecular mechanism that explains the significance of the MPK-IAA15 module in suppressing lateral root development in response to drought stress.

P-07-06

Upregulation of auxin receptor TIR1 in auxin autonomous plant cell proliferation

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Plants are characterised by extensive phenotypic plasticity. Plant cell identity is defined as a reciprocal interaction of transcriptional regulatory networks, epigenetic modulations, and the activity of plant signalling compounds. Auxin is one of the most important morphogenic molecule in plants. Together with other plant hormones, it induces cell proliferation and plays a crucial role in in vitro organogenesis. The response to auxin is regulated by spatiotemporally specific expression of the auxin signalling machinery and by precise regulation of auxin concentration through multiple biosynthetic and metabolite pathways supported by auxin transport involving influx, efflux, and intracellular transport. The rapid and steady proliferation of cells in plant cell cultures presents unique model for studying mechanisms involved in cell cycle regulation. Here we present metabolic and transcriptome analyses in the habituated auxin-autonomous BY-2 line (BY-2H). We uncovered increased auxin biosynthesis and decreased metabolism in BY-2H cells although levels of free IAA remained relatively stable. RNA-seq comparison revealed significant upregulation of the *NtTIR1T,* coding for a homolog of TIR1 auxin F-box receptor protein. Resequencing of the BY-2 and BY-2H genomes revealed substantial (> 150x) amplification of the *NtTIR1T* gene in BY-2H cells. Finally, the response to inhibitors of auxin signalling treatments suggested a BY-2H-specific compensatory mechanism based on upregulated TIR1.

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P-07-07*

Natural genetic variability in multistep phosphorelay as a tool for elucidating drought adaptation in *Arabidopsis thaliana*

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Molecular networks governed by plant hormones and external stimuli facilitate plant adaptation to changing climate conditions. Understanding the underlying mechanisms is key to developing climate-adaptable plant varieties allowing mankind to overcome challenges posed by severe weather unpredictability, diminishing arable lands, and a growing global population. Using the 1001 Genomes resource, we explored the natural variations in cytokinin-responsive histidine kinases AHK2, AHK3, and AHK4/CRE1. We identified accessions carrying single nucleotide polymorphisms (SNPs), associated with both up-and down-regulation of plant responsiveness to cytokinins, while not affecting the ability of the sensors to bind cytokinins. Drought stress responses of the accessions with altered sensitivity to cytokinin were analyzed using an automated plant phenotyping platform (WIWAMxy). In the presence of exogenous cytokinin, the accessions possessing higher cytokinin sensitivity sensed drought earlier and displayed a stronger reduction of growth under drought than Col-0. Compared to that, the accessions demonstrating lower cytokinin sensitivity appeared less drought-responsive. Our findings imply the existence of a mechanism allowing us to fine-tune the dynamics of the stress response by changing the sensitivity of hormonal signaling. The research has significant implications for the synthetic biology approaches aimed to increase crop resistance to abiotic stresses.

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P-07-08

Interplay of plant hormones and organic nitrogen in the modulation of root system architecture

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Nitrogen (N) represents a life-essential macronutrient and growth-limiting factor for plants. However, inorganic nitrogen (IN)-based fertilizers significantly contribute to harmful environmental consequences. On the other hand, very little is known about how organic N (ON), a more environmentally friendly source of N, regulates plant development. Our results show that ON in the form of the amino acid glutamine has a promoting effect on root biomass. ON modulates root system architecture by inhibiting primary root growth while significantly promoting lateral root (LR) branching. Remarkably, the adaptation of root growth to the change of N source from IN to ON resulted in highly synchronized LR development. This adaptation effect was strikingly different from the acropetal gradient of LR development after continuous IN treatment. Microscopic analyses revealed that the ON source greatly impacts LR primordia spacing, resulting in significantly higher LR organ density when compared to IN. In addition, our studies indicate that ON effect on root growth is mediated in concert with plant hormones. The levels of most auxin metabolites were significantly elevated in response to ON while the concentration of bioactive auxin was higher in ON-treated roots. In addition, IN treatment resulted in higher levels of trans-zeatin types of cytokinins, while ON induced the accumulation of cis-zeatin types. These novel findings indicate that auxin and CK metabolism is selectively altered in response to different N sources. This has raised new questions on how plants modulate their RSA in adaptation to different N environments.

P-07-09

Phytohormonal study of turion development in two aquatic carnivorous plants

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Specific overwintering organs – turions – are very common in subtropical to subarctic zones and are formed at least in 14 genera from nine plant families of aquatic vascular plants. Turions are vegetative, dormant and storage green organs formed in perennial aquatic plants in response to unfavourable ecological conditions (temperature decrease, shortening day length and/or reduction of daily light dose) mostly at the beginning of autumn. The aim of this study was to determinate and compare contents of endogenous levels of auxins, cytokinins and ABA in growing shoot apices (controls) and gradually developing turions of two model aquatic carnivorous plant species, Aldrovanda vesiculosa L. (Droseraceae) and Utricularia australis R. Br. (Lentibulariaceae) and correlate them with dark respiration and photosynthetic rates and contents of photosynthetic pigments and nitrogen (N) and phosphorus (P) in young trap-free leaves at the same time points and stages of turion development. On the basis of these results, more general ecophysiological knowledge was obtained about the development and maturation of turions focused on the role of the content of endogenous levels of phytohormones and photosynthesis for the formation of reserve substances and the rate of reutilization of N and P from senescent leaves. The selection of these two model species was based on the easy availability of dozens of individuals in common outdoor culture and also on the fact that many ecophysiological studies have been performed on these species, including their turions.

P-07-10

PILS6 sets the temperature-dependent oscillatory lateral rooting

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Plant root development is strongly influenced by temperature. High temperature (HT) has been shown to enhance primary root growth through increased auxin signalling (Feraru et al., 2019; Waidmann et al., 2020), but its impact on lateral root (LR) growth and development, as well as the underlying mechanisms involved, remain unclear. PIN-LIKES (PILS) proteins are putative auxin carriers located in the endoplasmic reticulum (ER) that limit nuclear auxin availability (Barbez et al., 2012; Beziat et al., 2017; Feraru et al., 2019). Our previous data indicated that HT enhances nuclear auxin signalling and primary root growth by decreasing the abundance of PILS6 proteins (Feraru et al., 2019; Sun et al., 2020). However, whether HT also affects LR growth in a PILS-dependent manner is unknown. Here we report that the *PILS6* loss and the overexpression of PILS6 increase and reduce lateral rooting when compared to the wild type, respectively. Moreover, we show that a moderate temperature increase (from 21°C to 25°C) increases the lateral root density, which is strictly dependent on PILS6. We also found that the ectopic activity of PILS6 disrupts the frequency of DR5 oscillations in the root tip. Our ongoing work hence focuses on the possibility that temperature regimes define lateral rooting by affecting auxin signalling oscillation in a PILS-dependent manner.

P-07-11*

How do PKS proteins regulate Arabidopsis phototropic bending?

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Understanding how plants reorient themselves towards a light source is of great importance for growing many economically important crops. Our comprehension of basic processes including responses towards directed blue light, phototropism, remains partial. How blue light perception by receptors called phototropins results in differential cell growth and eventually bending has been extensively studied in Arabidopsis hypocotyls, yet, we still know little about the underlying molecular mechanisms. Light-driven gradients in pH and auxin distribution that cause cell growth changes have been implied in the process, but how they are connected to phototropin signaling and coordinated on opposite sides of the hypocotyl remains elusive. Members of the Phytochrome Kinase Substrate (PKS) family have been proposed to act early in phototropin signaling. However, their mode of action remains unknown. Here, using BFA and NPA treatment in WT and *pks* mutants, we are testing whether PKS proteins might be involved in PIN-mediated auxin gradient formation. Our FM4-64 assay reveals that general endocytosis is not affected in *pks* mutants. However, they could be involved in specific targeting regulators of auxin transporters to PM. As a first step to address this question, we are using qDII lines to uncover possible role of PKS proteins in early auxin signaling. To test whether PKS proteins play a role in light-driven apoplast acidification, we analyzed phosphorylation of the penultimate Thr residue of AHA H^+ ATPase and tested fusicoccin sensitivity of *pks* mutants. Collectively, these experiments will inform us on the mechanism of PKS action during phototropic growth re-orientation.

P-07-12*

microRNA165 and 166 modulate salt stress response of the Arabidopsis root

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In plants, developmental plasticity allows for the modulation of organ growth in response to environmental cues. Being in contact with soil, roots are the first organ responding to soil abiotic stresses such as high salt concentration. In the root, plasticity relies on changes in the activity of the apical meristem, the region at the tip of the root where a set of self-renewing undifferentiated stem cells sustains growth. We show that salt stress promotes root meristem cells differentiation via reducing the dosage of the microRNAs miR165 and 166. By means of genetic and molecular analysis we show that the levels of miR165 and 166 respond to high salt concentration, and that miR165 and 166-dependent PHB modulation is fundamental for the response of root growth to this stress. Salt dependent reductions of miR165 and 166 causes rapid increase of the Arabidopsis homeobox protein PHABULOSA (PHB) expression and production of the root meristem pro-differentiation hormone cytokinin. Our data provide direct evidence of how the miRNA-dependent modulation of transcription factors dosage mediates plastic development in plants.

P-07-13*

Sweet shaping of root system architecture under dehydration stress

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Dehydration stress is one of the leading factors limiting crop yield globally. Plant roots display high plasticity and play a very crucial role in plant adaptation and response to dehydration stress. In this study, we report that glucose regulates root directional growth under high agar-induced dehydration stress via TOR signaling pathway. TOR RNAi seedlings were hyposensitive, whereas seedlings overexpressing *TOR* were hypersensitive to glucose induced root growth deviation under control and dehydration stress condition. Our analysis revealed that cytokinin signaling is a prerequisite for dehydration stress-induced straightening of roots. Furthermore, physiological and molecular assays showed that glucose signaling antagonizes cytokinin signaling via TOR under stress condition. Our study also revealed that glucose and cytokinin regulate root directional growth by modulating various components of auxin signaling and transport, particularly, auxin tranporter PIN7 seems to play a key role in dehydration stress-induced straightening of roots. Glucose induces asymmetric distribution of *DR5::GFP* across the root tip, whereas dehydration stress and cytokinin abolish the asymmetric distribution of *DR5::GFP*. Interestingly, high agar induced dehydration stress triggered loop formation in roots on horizontal growth conditions and poor seedling growth. Glucose could bring down loop formation in roots and induce a root architecture capable of better exploration of the surface of the media. Cytokinin and auxin signaling are also involved in this root adaptive response. Altogether, glucose-mediated change in root direction enhances the plasticity of root architecture and thus is an adaptive feature under dehydration stress conditions.

P-07-14

From single cell to root system shaping in response to nitrogen

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Roots system (RS) shows a high degree of plasticity requiring *de novo* organogenesis, which allows plants to develop new lateral roots (LRs) and thus adapt to external conditions. Generation of LRs is regulated by internal signals and external factors, such as phytohormones and nitrogen availability, respectively. Inorganic nitrogen (IN) is extensively overused despite its harmful effects on environment. Contrary, the role of organic nitrogen (ON), an abundant N source in the soil, in plant development has remained elusive. Preliminary results showed, Gln as a representative ON compound promotes LR development compared to IN in *Arabidopsis*. GATA23 is a transcription factor, which controls LR founder cell (FC) specification and that is expressed during the early stages of LR initiation. Interestingly, the p*GATA23:*GFP signal was observed not only at LR pre-branch sites, but also in xylem pole pericycle cells of the transition and elongation zone.

Hypothesis: 'Dormant' LRFCs generated in the apical part of the root could start developing into LRs, depending on environmental stimuli. ON, being such a signal, could promote programming of these dormant cells by blocking cytokinin-dependent inhibition, resulting in induction of LR organogenesis. The goal of our project is therefore i) to unravel how ON controls LR organogenesis and thus the adaptive capacity of the RS, ii) to decipher re-programming of RS architecture under ON treatment at the single-cell level and to understand the potential involvement of cytokinin in this process. Single-cell RNA-seq methods will be used to study p*GATA23:*GFP^{+} cells during early stages of FC specification.

P-07-15

Exploring the role of the NGR1 protein in plant root gravitropic response

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Plants can sense gravity and adjust their organs' growth direction accordingly. This phenomenon is called gravitropism. Recently the NGR/LZY family genes were identified as key regulators of root and shoot gravitropism in different plant species [1, 2]. Knocking out Arabidopsis NGR1,2,3 results in anti-gravitropic phenotype with the root growing upwards [3], PIN3 polarized to the upper cell sides, and auxin flow redirected to the upper side of the root after gravistimulation [4], while the amyloplast sedimentation in *ngr1,2,3* plants remains normal [5]. NGRs connect the sedimentation of amyloplasts in the columella cells and redistribution of the auxin flow toward the lower side of the root during the plant gravitropic response. Though, the exact mechanism of NGR's action remains unclear.

In our project, we explore the role of the NGR protein in plant gravitropism using a vertical stage confocal live imaging setup [6]. We investigate NGR's polar localization on the bottom side of the root columella cells and its re-polarization upon amyloplast sedimentation. Additionally, we clarify the mechanism of NGR's membrane association and its significance for the proper plant gravitropic response.

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P-07-16

Organ-targeted cold stress affects endogenous cytokinins in rice

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Rice (*Oryza sativa*), the world-wide cultivated crop, is sensitive to cold stress which limits its geographical distribution. Understanding of the mechanisms of stress responses will enable to enhance its tolerance. In this study, phytohormones, transcriptome, proteome and sugar content were evaluated in rice plants exposed to cold stress (5°C) targeted to leaves, roots or whole plants using special thermoregulatory vessels. Responses to cold stress to roots resembled those to stress to whole plants indicating important role of roots in stress responses. Targeted stress resulted during recovery in faster growth stimulation of the non-exposed organs which was connected with hormonal regulation.

Here, we demonstrate how cold stress affects cytokinin levels, metabolism and signalling. Cold stress in general downregulated levels of *trans*-zeatin, dihydrozeatin and isopentenyladenine in roots due to the necessity to supress plant growth and relocate the energy sources. Cold stress to roots inhibited cytokinin levels also in leaves probably due to diminished transport from roots. The most specific changes were found after stress targeted to leaves. This variant maintained cytokinin synthesis and transport from roots which was associated with photosynthesis protection and production of antioxidant enzymes. Higher levels of low active *cis*-zeatin and methylthio-derivatives of zeatin ribosides in roots of this variant correlated with supported growth of primary root, but they could be associated also with attraction of sugars and nutrients. In summary, organ-targeted stress responses reflect the individual roles of each organ.

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P-07-17

Auxin and cytokinins response to arsenic toxicity in tubers of cherry radish

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Soil contamination with arsenic (As) poses a threat to plants because it disrupts the biological function of plants by inhibiting their growth and developmental process. Phytohormones, as chemical messengers, can improve the tolerance of plants to various stresses, however, their role in plant response to As has not been fully elucidated. Hence, we aimed to evaluate the effect of As on endogenous auxin and cytokinin (CK) changes in tubers of cherry radish (*Raphanus sativus* var. *sativus* Pers.) cultivated under low and high soil contamination (20 and 100 mg As/kg soil). Indole-3-acetic acid (IAA), the main auxin in plants, was increased at low As level and decreased at high As compared with the control. In contrast, tryptophan, a precursor in most IAA biosynthetic pathways, was decreased by both As levels. In the case of CKs, the effect of both As concentrations varied among individual CKs. Dihydrozeatin and its related metabolites were not determined in tubers. Several CKs declined due to high As level, however, only a group of CK phosphates (*trans*-zeatin riboside monophosphate, *cis*-zeatin riboside monophosphate, isopentenyl adenosine monophosphate) showed a significant decrease. A decrease in this group of CKs was also observed at a low As level. Of the CK O-glucosides group, only *cis*-zeatin riboside-O-glucoside was detected. Auxin and CK responses indicate high variability in the metabolic response of cherry radish to As, with high As strongly affecting tubers.

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