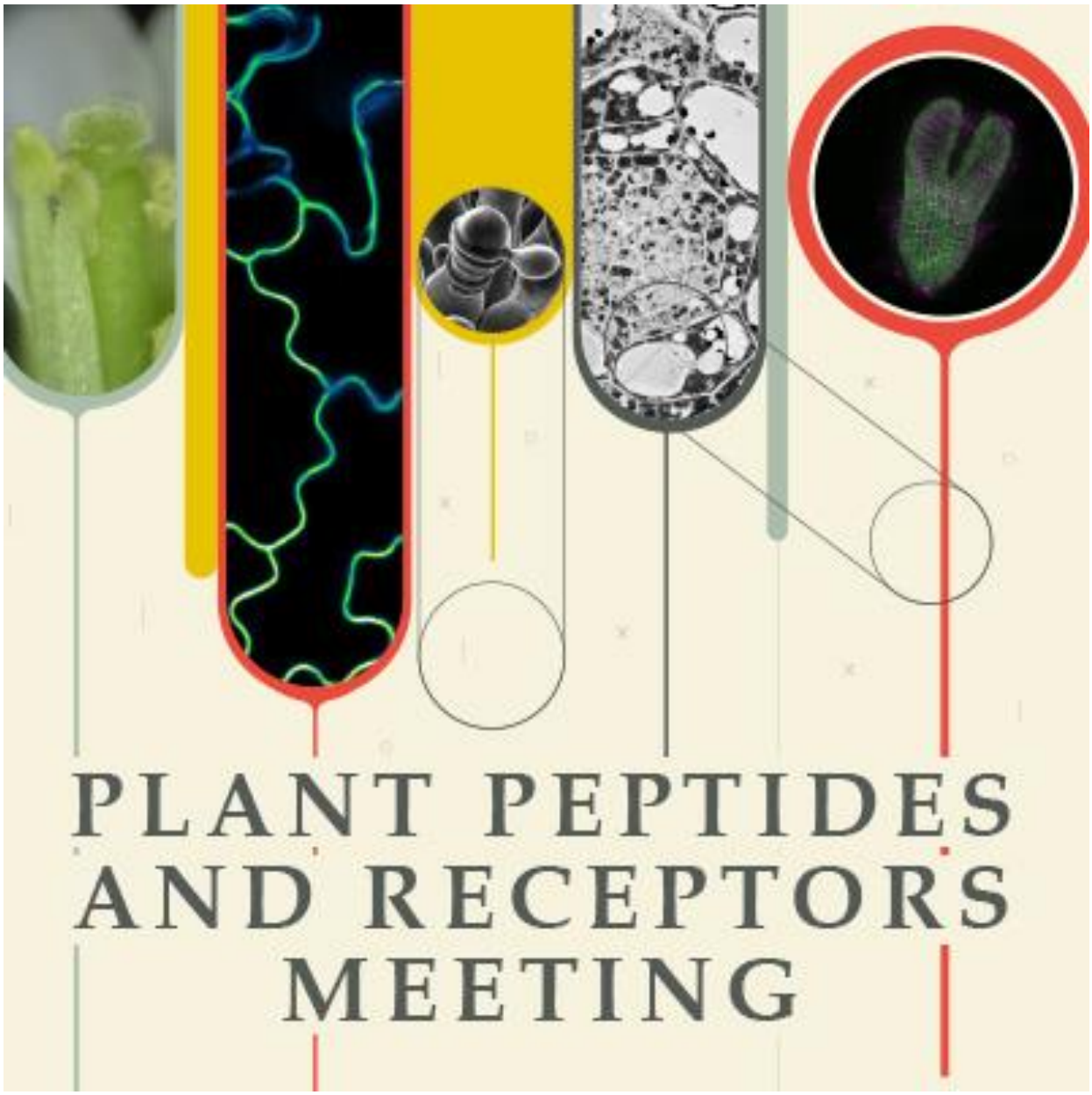
Abstract Booklet



2-4 Oct 2023

Lyon, France

Content

Imaging Approaches for Peptide Signaling in Pollen Tube Guidance

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In flowering plants, precise directional control of pollen tubes in the pistil, namely, pollen tube guidance, is critical for successful sexual reproduction. Pollen tube guidance involves complex peptide signaling. Response of pollen tubes to peptide ligands is quick, therefore studying pollen tube guidance potentially provides us an opportunity to reveal spatio-temporal dynamics of molecules and molecular signaling. However, there exist technical difficulties in live imaging of pollen tube guidance. We have been trying to develop imaging technologies to study pollen tube guidance, by using two genera, *Torenia* and *Arabidopsis*, as biological models. One direction of imaging approaches is deep-tissue imaging in the pistil tissue. By two photon microscopy, we examined cellular dynamics and signaling in one-to-one guidance between multiple pollen tubes and multiple ovules (Mizuta et al., 2023, bioRxiv). Ovules were suggested to emit multiple signals for pollen tubes, including an integument-dependent directional signal that reaches the inner surface of the septum and adhesion signals for emerged pollen tubes on the septum. Not only FERONIA in the septum but ovular gametophytic FERONIA and LORELEI, as well as FERONIA- and LORELEI-independent repulsion signal, were involved in polytubey blocks on the ovular funiculus. These funicular blocks were not strictly maintained in the first 45 min, explaining previous reports of polyspermy in flowering plants.

Another direction of imaging approaches involves super-resolution and single-molecule imaging of cultivated pollen tubes. Chemotropism and tip-growth of pollen tubes depend on receptor-like kinases on the plasma membrane of pollen tubes and their peptide ligands. For example, defensin-like LURE attractant peptides and LURE-type attractant peptides are secreted by two synergid cells on the side of the egg cell. LURE1 peptides are received by PRK6 receptor, which is also involved in pollen tube growth. On the other hand, ANX and BUPS receptors have been suggested to monitor integrity of the pollen tube by RALF autocrine signaling pathway. We previously demonstrated PICALM5 specifically internalizes ANX but not PRK6 at the subapical region of the pollen tube (Muro et al., 2018, Commun. Biol.). Such precise spatio-temporal controls of receptors in membrane traffic might be critical for pollen tube guidance. Our recent challenges in super-resolution/single-molecule imaging would be introduced.

Phytocytokine signalling at the plant-environment interface

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In response to environmental cues plant cells actively produce and secrete a diverse a range signalling peptides into the apoplast. These peptides play a crucial role in modulating cellular responses in cells expressing their corresponding receptors. In the context of biotic stress, these signalling molecules are commonly referred to as phytocytokines. Here I will present our recent advances in understanding of two recently described phytocytokine signalling modules.

SERINE-RICH ENDOGENOUS PEPTIDES (SCOOP) peptides were originally described as a 14- member family however recent publications have revealed this family is larger than previously thought. Here we present a comprehensive annotation of *PROSCOOP* peptides within the Col-0 genome identifying 50 putative PROSCOOP peptides. Intriguingly, despite their diverse sequences, all active SCOOP peptides exhibit MIK2-dependent activity. Building on this I will present our recent work on the biogenesis of these SCOOP peptides.

In addition, we will provide insights into ongoing work to understand the physiological role of the recently described HSL3(NUT)-CTNIP(SCREW) signalling module.

Immune receptor signaling at plasmodesmata: similar but different

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The plant immune system relies on the extracellular perception of molecules that signal the presence of a microbe threat, with one response being the closure of plasmodesmata that connect neighbouring cells. We previously found that chitin and flg22 trigger specialized immune signaling cascades in the plasmodesmal plasma membrane, and that these are executed independently of receptor signalling in the rest of the plasma membrane. However, both flg22 and chitin trigger plasmodesmal closure via callose synthesis suggesting that the signaling pathways ultimately converge at or upstream of this process. To establish the hierarchy of signaling at plasmodesmata and identify points of convergence, we profiled the dependence of plasmodesmal responses triggered by different elicitors on a range of plasmodesmal signaling machinery. Thus, we identified that microbial elicitors and endogenous defence hormones trigger signalling cascades that converge at a plasmodesmata-specific complex that relays activation of the callose synthesis. How the machinery to execute these signalling cascades is specifically recruited to plasmodesmata remains an important question and our preliminary investigations suggest membrane domain proteins differentially sort chitin receptors to the plasma membrane and plasmodesmata to define receptor signalling domains. These domains offer a possible mechanism by which plasmodesmal signalling is regulated independently from other immune responses, leading us to hypothesise that plasmodesmata might not always close when a cell activates immune responses.

Plant adaptation to fluctuating nitrogen environments by long-range mobile peptide signals

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Nitrogen (N), which is mostly present as nitrate in soils, is an essential macronutrient that plays a crucial role in plant development. However, due to the high mobility of nitrate ions through soil with water, marked spatiotemporal fluctuations can occur with respect to soil nitrate availability. To cope with such fluctuations in the external N environment, plants have evolved regulatory mechanisms that enable them to modulate the efficiency of root N acquisition in response to their internal N demand and rhizosphere N availability. This systemic adaptive response is mediated by shoot-root communication via phloem-mobile descending CEPD1/2/CEPDL2 polypeptides. Among these, CEPD1 is induced in shoots in response to the peptide hormone CEP, which is a root-derived N deficiency signal, while CEPDL2 is induced in response to the shoot's own N deficiency. CEPD2 is induced in response to both root-derived CEP and shoot N deficiency. CEPD1/2/CEPDL2 promote nitrate uptake in roots by both transcriptional upregulation of high-affinity nitrate transporter genes, such as *NRT2.1*, and dephosphorylation-mediated post-translational activation of NRT2.1 by the protein phosphatase, CEPH, which is also induced by CEPD1/2/CEPDL2. We are currently using biochemical and reverse-genetic approaches to dissect the molecular mechanisms through which CEPDs specifically regulate genes involved in nitrate uptake. Possible targets of the CEPD pathway will be discussed.

Medicago truncatula SOBIR1 controls specificity in the Rhizobium-legume symbiosis

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The Rhizobium-Legume (RL) symbiosis is an intimate interaction that results in huge benefits to host plants in terms of nitrogen nutrition. In the *Medicago truncatula* model legume, SUPPRESSOR OF BIR1 (MtSOBIR1) was identified as an interactor of NOD FACTOR PERCEPTION (MtNFP), a lysindomain Receptor-Like Kinase that plays a key role in the RL symbiosis. In addition to this symbiotic role, MtNFP is involved in plant immunity, and as such might be a component of different receptor complexes that perceive distinct signals leading to corresponding responses. We showed that the kinase domain of MtSOBIR1 is active and can transphosphorylate the pseudo-kinase domain of MtNFP. Like in other plants, our data suggest a positive role of MtSOBIR1 in immunity; *MtSOBIR1* could functionally complement an *Atsobir1* mutant for defence activation, and a *Mtsobir1* mutant was defective in pathogen-induced defence gene expression. We also showed that *MtSOBIR1* has a symbiotic role with *Mtsobir1* mutants showing strong symbiotic phenotypes in a plant genotype- and rhizobial strain-specific manner. Together, these data suggest that MtSOBIR1, like MtNFP, has a role both in symbiosis and in plant immunity.

Which side are you on? Linking polarized receptor kinases to root cell division control

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Understanding the mechanisms that activate and repress cell division in multicellular eukaryotes is a fundamental question in developmental biology that is directly tied plant productivity. In plants, cell division activity and division plane orientation are critical to tissue form and function and directional signaling is frequently proposed to have a key role in coordinating these developmental processes. In the Arabidopsis root, with its precise organization of cells and tissues, my lab investigates how polarized transmembrane receptor kinases modulate the timing and orientation of cell division. We have identified, KINASE ON THE INSIDE (KOIN), which represses cell proliferation in the root's longitudinal axis and restricts root width. KOIN-GFP unexpectedly accumulates only in the interior cell types, including the endodermis where it localizes to the inner polar domain. In addition, closely related RLKs, INFLORESCENCE AND ROOT APICES KINASE (IRK) and PXY/TDR CORRELATED 2 (PXC2) function to repress endodermal cell divisions specifically in the root's radial axis and also restrict root width. In contrast to KOIN, IRK and PXC2 are localized to the outer polar domain in the endodermis. Endodermal-specific expression of KOIN or IRK is sufficient rescue their respective mutant phenotypes, suggesting non-cell autonomous downstream functions. We propose directional signaling pathways converge on the endodermis where polarized receptor kinases, KOIN and IRK/PXC2, inform cell division activity and root width to mediate coordinated organ growth and development.

The IDA signaling pathway: cell separation and biotic stress responses.

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Plants shed organs through the process of abscission by responding to endogenous signals and by monitoring environmental cues like biotic and abiotic stresses. Integrating these signals allows plants to abscise organs in a timely manner. In Arabidopsis floral organ abscission serves as a model to study the genetic control of this process. The INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide is secreted in abscission zones where it is recognised by the receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2) and co-receptors belonging to the somatic embryogenesis receptorlike kinase (SERK) family. Upon IDA perception, the receptor complex triggers a downstream phosphorylation cascade of mitogen-activated protein kinases (MAPKs) that further induce transcriptional reprogramming via knotted-like from Arabidopsis (KNAT) transcription factors. The molecular effectors helping the HAE/HSL2-SERK complex relay the signal onto the MAPK cascade have not been identified.

Here we present recent progress in the identification of the BRASSINOSTEROID SIGNALING KINASE (BSK) family as a RLK-mediated signaling cascade driving floral organ abscission. We will also present results on how the transcriptional regulation of *IDA* allows for integration of biotic stress and abscission.

Arabidopsis LRR VIII-2 Receptor-Like Kinases regulate intra- and inter-species pollen-pistil interactions

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In Arabidopsis, the regulation of pollen-pistil interactions begins rapidly after pollen grains have landed on the stigmatic papillae at the top of the pistil. As the pollen grains are in a desiccated state for dispersal, the first post-pollination step is for the pollen grains to hydrate. The water for hydration comes from the stigma but is only released when the pollen grain is recognized as compatible. Following hydration and germination, a pollen tube emerges and penetrates the stigmatic surface to begin its journey down the reproductive tract to an ovule for fertilization. In *Arabidopsis*, many signalling players have been identified that regulate the later stages of these pollen-pistil interactions (e.g. ovular pollen tube guidance and reception), but less is known about the regulators of these interactions in the preceding stages. Through mutant analyses, we have identified *Arabidopsis Receptor-Like Kinase (RLK)* genes in the LRR VIII-2 subfamily that play an essential role in the upper pistil to promote compatible pollen hydration and pollen tube growth. In addition, we uncovered a role for these RLKs in the stigma to reject interspecies pollen when closely *Capsella rubella* pollen was used to pollinate these *LRR VIII-2 RLKs* mutants. To better understand the function of these LRR VIII-2 RLKs in the pistil, a yeast two-hybrid screen was conducted with one member, RKF1, and several interactors were identified. Further mutant analyses supported a role for one group of these interactors in the pistil in response to compatible pollinations. Thus, these *Arabidopsis LRR VIII-2 RLKs* play a dual role in the female pistil to promote compatible pollen and block closely-related interspecies pollen, and further research is underway to uncover the cellular responses activated by these RLKs.

From a Proteomic Dataset to Characterization of a Novel Plant Peptide

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Despite their recognition as key players in defense, development, physiology, and cell-to cell communication, thus far only a few peptides have been discovered and functionally characterized in crop plants. Recent proteomic and transcriptomic studies on different stages of maize male flower development implicate peptides as coordinators of growth and cellular behavior. Therefore, we aimed to identify and characterize novel peptides that are involved in plant cellular development. To this end, we selected 39 CANDIDATEs (CANs) from maize proteomic data and screened them with the recently established *Ustilago maydis*-based Trojan Horse approach. Here, we present *CAN25*, a gene that encodes for a so far undescribed secreted peptide. CAN25 contains a 14-amino-acid sequence highly conserved throughout land plants. Strikingly, CRISPR/Cas9 mediated knock-out of *can25* revealed homozygous lethality in *Arabidopsis thaliana*. Siliques of heterozygous *can25* +/- plants yield approximately 25% seeds with abnormal characteristics indicating a defect during embryogenesis. However, the cellular patterning of the embryo seems to be undisturbed, but the growth rate is reduced which ultimately leads to the abortion of the developing seed. In combination with other preliminary data from our lab this indicates that the newly discovered secreted peptide CAN25 might be involved in growth regulation signaling in plants. We will show latest results on the characterization of CAN25 in *A. thaliana* and its homolog in *Zea mays*.

The Pollen Tube Highway - Functional characterization of novel CRPs during fertilization in Zea mays

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Small polymorphic proteins, being secreted from pollen/pollen tubes, embryo sacs or sporophytic maternal tissues, often facilitate species-specific interactions to overcome pre-zygotic hybridization barriers in plants. The most well-known group of small polymorphic proteins involved in fertilization processes are small cysteine-rich peptides (CRPs). We are investigating novel CRPs that are highly expressed in the reproductive tissues of *Zea mays* to elucidate their roles in pollen adhesion, hydration, germination as well as pollen tube growth and guidance culminating in sperm cell release. These novel CRP candidates include *ZmBBI1-3*, containing four cysteine residues and being highly expressed in pollinated/infected silk. *ZmBBI1-3* belong to a family of 17 *ZmBBI* members in maize. Furthermore, *ZmLTP1.6* and *ZmLTP3*, belonging to a family of 21 *ZmLTP* members, are strongly expressed in silk/hair cells and contain eight conserved cysteine residues. Moreover, *ZmPOE1.1* and *ZmPOE1.2* are highly expressed in pollen tubes and belong to a family of 26 *ZmPOE* members with six conserved cysteine residues. A further CRP (*ZmCRP*) with four cysteine residues is a single copy gene in maize and does not occur in genomes of other plant species. To gain knowledge about the function of these candidates during fertilization in *Zea mays, in-vitro* pollen tube germination experiments were performed using pollen from RNAi- as well as CRISPR/Cas9-edited plants. Pollen tubes of various mutants were less stable compared with those from wild-type plants and partly showed proposed hydration defects. Additionally, wild-type pollen germinating on mutant silk of the *ZmLTP1.6* RNAi-line show growth abnormalities. Subcellular localization analysis showed that ZmPOE1.1 and ZmPOE1.2 were localized in the cytosol and cell wall, while ZmLTP1.6 and ZmCRP can be found in endosomes and other vesicles. In conclusion, ZmPOE1.1, ZmPOE1.2, ZmLTP1.6 and ZmCRP are localized in different compartments along the protein secretion pathway. We are currently investigating the function of these candidates and additionally aim to identify possible interaction partners to gain knowledge about the underlying molecular processes regulated by these candidates.

Capturing 3-hydroxy fatty acid: Characterization of the immune receptor LORE in Brassicaceae

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Plants evolved cell-surface pattern recognition receptors to detect signs of danger – such as microbe-associated molecular patterns (MAMPs) – in the apoplast and activate defense responses. Medium chain 3-hydroxy fatty acids (3-OH-FAs) from Gram-negative bacteria are sensed as MAMPs by the S-domain (SD) receptor kinase LORE (alias SD1-29) in *Arabidopsis thaliana*. Being phylogenetically restricted receptor kinases in Brassicaceae, whether other species in the same family as Arabidopsis have LORE orthologs and how the orthologs perceive 3-OH-FAs is unknown. Therefore, we explored the natural diversity of 3-OH-FA-triggered responses in different Brassicaceae species and assessed the interaction of 3-OH-FAs with putative LORE orthologs. Though the responsiveness to 3-OH-FAs is diverse within Brassicaceae, the tested LORE orthologs can bind 3-OH-FAs. Interestingly, not all LORE orthologs rescue the function of sensing 3-OH-FAs in *A. thaliana lore-1* mutant, indicating that multiple factors, including ligand-binding and kinase activity, determine LORE-dependent immune signaling. Furthermore, the 3-OH-FA binding structure on LORE was identified by *in silico* and *in vitro* approaches. Overall, our findings shed light on the mechanism of LORE-mediated 3-OH-FA sensing in Brassicaceae.

CEP signaling coordinates cell surface immunity with nitrogen limitation

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Emerging evidence suggests that plants employ endogenous peptides to coordinate plant growth with immunity. Immuno-modulatory peptides were classified as phytocytokines and are able to either amplify or attenuate the strength of immune response. We identified specific members of the C-TERMINALLY ENCODED PEPTIDE (CEP) family as novel immune-modulatory peptides in *Arabidopsis thaliana*. CEP application triggers hallmark pattern-triggered immunity (PTI) signaling outputs. Propeptide overexpression and loss of function studies confirmed a critical role of CEPs in immunity against infection by *Pseudomonas syringae* pv. *tomato*. CEPs are predominantly expressed in roots, but we show that leaf expression of CEPs is required for their immune-regulatory function. CEPs are generally perceived by the leucine-rich repeat receptorlike kinases CEP RECEPTOR 1 (CEPR1) and CEPR2. We now show that CEP4 also binds to and signals through the closely-related RECEPTOR-LIKE KINASE 7 (RLK7). All three CEP receptors show distinct expression patterns and feed to CEP-mediated responses with different specificities. This suggests tissue-specific contributions of individual receptors to CEP-mediated immunity. Knocking out all three receptors leads to an abolishment of CEP-induced defense responses. In accordance to the role of CEPs in nitrogen (N) starvation, we found that reduced N availability promotes PTI signaling in a CEP-dependent manner. This indicates that CEPs play a central role in balancing the cross-talk between the plant's N status and immunity.

Characterization of a small family of systemin-like peptides in Solanum lycopersicum

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Systemin was discovered as the first plant hormone-like peptide in tomato plants (*Solanum lycopersicum*). The 18-mer peptide acts as a phytocytokine that is perceived by the LRR-RLK SYR1 at the cell surface to mediate defense responses against insect herbivores. Systemin signaling triggers the production of jasmonates at local wound sites and leads to the expression of an array of defense genes, both locally as well as in distal tissues. It remains unclear how systemin signals are transmitted and integrated into the plant immunity network. In the current study, we identified three new prosystemin genes giving rise to systemin-like peptides B, C and D. Employing transcriptomic approaches, we investigated tissue responses to exogenous application of these systemin-like peptides compared to canonical systemin. Our time-series transcriptome datasets demonstrate levels of generality and specificity of genetic components involved in defense responses, and provide insights into the spatio-temporal regulation of transcriptional re-programming by systemin analogs. The most profound changes in gene expression were observed in response to systemin C, including a suite of genes that was regulated in a SYR1-independent manner. Systemin and systemin-like peptides also differed in the extent to which they involve the phytohormone ethylene in downstream signaling. This study extends our current view of systemin signaling from single gene effects to that of a gene family-regulated network.

CLE peptide signaling regulates induced volatile emissions in maize

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Plant volatiles are critical factors shaping plant-biotic interactions. The biosynthesis and ecological functions of plant volatiles are well understood. However, how foliar volatile emissions are regulated remains unclear. Here, we present first evidence for a role of CLE (CLAVATA3/EMBRYO SURROUNDING REGION-RELATED) peptide signaling in suppressing stomata governed maize volatile release. In the absence of herbivory, the green leaf volatile (Z)-3-hexenyl acetate (HAC) increases volatile production and emission from maize leaves. We find this process is accompanied by reduced expression of genes encoding for the CLE peptide precursors ZmCLE1b3, ZmCLE1e9 and genes encoding their potential receptor ZmBAM1a. Exogenous supply of Zm-CLE1e9, but not ZmCLE1b3, inhibits HAC-triggered volatile release, suggesting ZmCLE1e9 as a negative regulator of volatile emissions. Such inhibition effect disappears in the *Zmbam1a* mutant, indicating ZmBAM1a as a potential receptor for ZmCLE1e9. Molecular docking supports ZmCLE1e9 and ZmBAM1a as a ligand-receptor pair. In good agreement with the peptide feeding assays, *Zmcle1e9* and *Zmbam1a* mutants both show increased volatile emissions upon HAC exposure. Intriguingly, ZmCLE1e9 does not suppress volatile biosynthesis. It reduces volatile emission by limiting stomatal aperture. In summary, our results suggest that HAC signaling downregulates ZmCLE1e9 signaling for maintaining stomatal aperture to facilitate volatile emissions.

Evolution of SERK-mediated specificity in plant development and immunity

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Many plant physiological processes are triggered by extracellular signals which are perceived through cell-surface receptors. Leucine-rich repeat receptor kinases (LRR-RKs) comprise the largest group of membrane receptors in plants and are involved in developmental and immune processes. Many LRR-RKs require the association with a complementary co-receptor from the somatic embryogenesis receptor kinase (SERK) family for high-affinity ligand binding, phosphorylation, and initiation of downstream signalling. Phylogenetic analysis showed that SERKs co-receptors form two clades in eudicots, SERK1/SERK2 and SERK3/SERK4. In *Arabidopsis thaliana*, these two clades play overlapping and specific roles. Thus, SERKs appear to contribute to LRR-RKs signalling specificity. In contrast, the evolutionary model plant *Marchantia polymorpha* only encodes one SERK ortholog belonging to the SERK1/SERK2 clade and whose function is currently not known. Orthologs of several SERK-associated receptors are present in all land plants whereas others emerged in different vascular plant lineages, coincident with SERKs diversification. Our project aims at understanding how LRR-RKs signalling pathways evolved in land plants and which are the molecular basis for SERK-mediated specificity. First, we will characterize the role of the *Marchantia* SERK and its putative associated receptors and compare them with their *Arabidopsis* counterparts. Then, we will perform comparative phylogenetic analyses between orthologous (co)receptors from all available plant genomes to identify molecular signatures for LRR-RK/SERK adaptation. Subsequent functional analyses in *Marchantia* and *Arabidopsis* will be key to unveil the determinants for SERK-mediated specificity at the molecular and functional level in an evolutionary context.

From an ancestral viral infection of Zea to the release of Zip1 – a maize specific phytocytokine

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Numerous small peptides belonging to different plant families have been identified and functionally characterised as signalling molecules in plants. These small peptides - termed phytocytokines - have been shown to be important in the regulation of plant development, reproduction, immunity and adaptation to environmental stress[1]. Phytocytokines are actively released via proteolytic processing from a pro-peptide and need to reach the apoplast upon pathogen attack to alarm bystander cells[2,4,3].

This project focusses on expression, processing and release of *Zea mays* Immune Peptide 1 (Zip1), derived from the propeptide PROZIP1. Our studies show that *prozip1* resides in the terminal repeat of a retrotransposon and emerged *de novo* in the genus *Zea*; prior human domestication[5]. PROZIP1 expression is tightly timed upon SA- and Zip1 treatment, but also cellular damage and recent experiments suggest, that the promoter of *prozip1*, located in chromosome 7, might contain specific features required for the SA responsiveness. Zip1 is a 17aa peptide involved in the activation of salicylic acid (SA) signalling, which further activates papain-like cysteine proteases (PLCPs) and induces PR gene expression to enhance the SA immune response[6].

PROZIP1 carries eight cleavage sites, which are likely targeted by various types of endopeptidases located in different cell compartments. Our experiments suggest that intracellular leupeptin-sensitive proteases may play an important role in the initial PROZIP1 processing steps, whereas PLCPs are essential for peptide clearance in the apoplast.

Summarizing, we show that maize ancestor teosinte utilised viral genomic material to generate a very defined and functional phytocytokine, reversing a pathogen attack to an immune defence strengthening maize immunity.

Ongoing experiments plan to provide better insights in involved proteases and a potential receptor by RNAseq and to monitor the release of Zip1 to the apoplast utilizing ELISA and MS.

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Growing Strong or Fighting Back: One MAPK Kinase Kinase to Balance It All

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The Mitogen-Activated Protein Kinase (MAPK) cascade, a vital signalling pathway in plants and other eukaryotes, plays a crucial role in integrating growth and immune signalling to optimize resource allocation. This cascade typically involves a MAPK kinase kinase (MKKK), a MAPK kinase (MKK), and a MAPK. While all three kinase families have expanded in plants, MKKKs represent the most diverse tier, with 80 members, many of which remain uncharacterized. Among these MKKKs, the membrane-associated MAP kinase kinase kinase 7 (MKKK7) was shown to play a role in modulating plant immunity in a phosphorylation dependent manner, with t-DNA knockdown mutants of *MKKK7* exhibiting increased resistance to *Pseudomonas syringae* DC3000 infection (Mithoe et al., 2016). Recent findings also identify MKKK7 as a differentially phosphorylated protein in a *map auxin responsive kinase/raf (mark/raf)* null mutant, suggesting its involvement in ultra-rapid auxin response signalling (Kuhn et al., 2022 bioRxiv). In addition to its intricate phosphorylation pattern, we found that *mkkk7* mutants display a reduced root growth phenotype characterised by fewer cells in the root meristem. Basal immune signalling however is unaffected in the mutants, when grown under sterile conditions. This prompted us to explore the underlying signalling pathway governing this growth phenotype. Through a comprehensive literature search, comparing both growth and immunity phenotypes, and immune precipitation-mass spectrometry data we propose that MKKK7 acts downstream of the phytosulfokine receptor PSKR1. PSKR1 recognizes the 5 amino acid peptide hormone Phytosulfokine, which promotes growth, cell expansion, and attenuates SA-signalling. We show in protoplast assays that *mkkk7* mutants are compromised in PSK-induced expansion. Subsequent RNA-Seq and RT-qPCR analysis confirmed that PSK-induced gene expression is attenuated in *mkkk7*. Finally, we found that *mkkk7* mutants show a hypersensitive immune response to commensal *Pseudomonas fluorescens* WCS365, indicative for elevated SA-signalling and linked to disruptions in the phytosulfokine pathway (Song et al., 2022 bioRxiv).

In summary, our findings suggest that MKKK7 acts downstream of the phytosulfokine receptor PSKR1 to modulate growth and attenuate SA-mediated immune signalling. We are currently investigating the complex phosphorylation pattern of MKKK7 and downstream signalling components in response to PAMPs and PSK to gain a better mechanistic understanding of the crosstalk in the signalling network used during plant hormone, peptide, and immune signalling.

Mithoe et al., 2016; 17(3): 441{454. EMBO Reports
Kuhn et al., 2022; bioRxiv (https://doi.org/10.1101/2022.11.25.517951)
Song et al., 2022; bioRxiv (https://www.biorxiv.org/content/10.1101/2022.11.07.515115v1)

MIK2 RLKs generally act as Fusarium elicitor receptors in diverse plant families

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In order to respond to environmental cues like the presence of potentially harmful microorganisms or abiotic stresses, plants have evolved a vast variety of cell surface-localized receptors. These can perceive exogenous danger signals including conserved structures of microbial origin or endogenous signaling molecules that often serve a regulatory function.

An exceptional receptor in terms of ligand diversity is the leucine-rich repeat receptor kinase MIK2. It is not only the receptor for the large family of plant endogenous SCOOP peptides (Rhodes et al. 2021, Hou et al. 2021), but it is also indispensable for immunity responses to elicitor preparations from different *Fusarium* and related fungi (Coleman et al. 2021). Interestingly, SCOOP peptides and SCOOP sensitivity have hitherto only been found in members of the *Brassicaceae*. However, species from other plant families, including tomato, soybean, and barley show immunity responses when treated with the *Fusarium* elicitor and they have genes that code for proteins with phylogenetic origin similar to MIK2 from *Arabidopsis thaliana*.

Through heterologous expression of putative MIK2 orthologues, we identified several proteins from different plant families that could confer Fusarium elicitor sensitivity to otherwise insensitive *Nicotiana benthamiana*. However, only MIK2 orthologues from members of the *Brassicaceae* could confer *At*SCOOP sensitivity, while this was not observed for any receptor cloned from other plant families. Similarly, treatment with the *Fusarium* elicitor, but not *At*SCOOPs, induces the formation of receptor complexes between putative MIK2 orthologues and immunity-related SERKs from the respective non-*Brassicaceae*. Our findings about MIK2 orthologues are further supported by a specific interaction observed between their ectodomains and the elicitor activity found in our *Fusarium* preparations. Collectively, the data supports that MIK2 RLKs generally act as *Fusarium* elicitor receptors in diverse plant families and SCOOP receptor function might have evolved as an additional function only in *Brassicaceae*.

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Peptides derived from insect egg-shells are perceived as signals of imminent herbivore attack

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Insects and their plant hosts have co-evolved over the last 0.5 billion years. Their intimate interaction has been recorded in numerous fossils, many of which document the deposition of insect eggs onto and into plant tissues. This reproductive strategy is advantageous to herbivorous insects and detrimental to their plant hosts, as o spring hatches in direct proximity to a preferred plant food source. Plants, in turn, have evolved mechanisms to actively defend against these future attackers in a pre-emptive strike, either by killing the insect eggs or by impairing hatching. For some plant-insect interactions, specific egg-derived danger signals have been shown to alarm the immune system of the respective plant host to the presence of the eggs. However, pairs of egg-derived ligands and their cognate immune receptors remain to be reported. Here, we report that peptides derived from the egg-shells of unrelated insect species are highly immunogenic in *Arabidopsis*. In addition, such peptide preparations from *Pieris brassicae* are recognized by diverse Angiosperms lineages, indicating that the ability of the plant immune system to perceive egg-shell peptides may be an ancient defense strategy. Current work aims to identify the immunogenic egg-shell peptide(s) and cognate plant immune receptor(s).

The Arabidopsis TNL CONSTITUTIVE SHADE AVOIDANCE 1 (CSA1) reveals activation of PTI and ETI responses downstream of the pattern recognition co-receptor BAK1

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Arabidopsis BAK1/SERK3 is known to mediate pattern-triggered immunity (PTI) as a coreceptor of leucine-rich repeat (LRR) pattern recognition receptors (PRRs) in plants. Genetic inactivation or overexpression of BAK1 and BAK1-interacting receptor-like kinases (BIRs) leads to deregulated cell death. Our recent work identified the TIR-NBS-LRR protein (TNL) CONSTITUTIVE SHADE AVOIDANCE required to regulate cell death downstream of BAK1. We discovered that CSA1 physically interacts with BIR3 but not directly with BAK1 and is required for autoimmunity initiated in *bak1 bir3* mutants. The effector HopB1 cleaves activated BAK1 and overexpression of HopB1 induces cell death in a CSA1-dependent manner. Moreover, cell death caused by the microbial pattern pg23, but not typical PTI responses, are CSA1 dependent. These findings highlight the role of CSA1 in guarding the homeostasis of BIR3 and BAK1 and activating cell death pathways initiated by both, patterns and effectors. Classical PTI responses are not altered in csa1 mutants while immunity is impaired, suggesting that both, PTI and CSA1-mediated ETI, pathways are activated downstream of BAK1 to ensure robust plant immunity. In the presence of BAK1 and BIR3, CSA1 is repressed and autoimmune cell death is kept under control. Here, we will present novel data on how BAK1 integrity is perceived by CSA1 and how ETI and PTI responses are integrated downstream of BAK1.

The receptor kinase MIK2 links cell wall integrity sensing to pattern-triggered immunity

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Plants employ plasma membrane-localized receptors to perceive non-self or altered-self. As part of this surveillance system, plants monitor their cell-wall integrity (CWI). Our group has previously identified the leucine-rich repeat receptor kinase MIK2 as being important for the perception of cell wall damage (CWD) induced upon cellulose biosynthesis inhibition. MIK2 also regulates immunity to diverse pathogens and pests, and is the receptor for the family of SCOOP phytocytokines. How MIK2 contributes to CWD response is however still unclear. Notably, we report here that treatment with the inhibitor of cellulose biosynthesis isoxaben (ISX) activates transcriptional reprograming largely overlapping with pattern-triggered immunity (PTI) responsive genes, suggesting a cross-talk between CWI and immunity. Accordingly, we found that CWD promotes PTI responses. Analysis of mutants impaired in CWI indicates that MIK2 is the key regulator for ISX-induced transcriptional reprograming. Consistently, CWD-promoted PTI responses are also mostly dependent on MIK2. Interestingly, we found that specific SCOOP peptides that are highly induced upon CWD are also required, suggesting they activate MIK2 to induce CWD responses and promotion of PTI. Collectively, our data indicate that the SCOOPMIK2 signaling module links CWI sensing and immunity in Arabidopsis.

(Receptor) Kinase signaling modules involved in regulation of nitrate transporters

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Nitrogen (N) is an important nutrient for plants, involved in processes such as nucleic acid and protein synthesis. Plants take up nitrogen in two forms, nitrate (NO3-) and ammonium (NH4+) via specific transporters in the plasma membrane. It is well known that nitrate transporters, but also ammonium transporters are regulated by phosphorylation. For example, in nitrate transporter NRT2.1 – the major high affinity nitrate transporter in Arabidopsis – activating and inactivating phosphorylation sites were discovered in the N- and C-terminus. However, the signaling modules and especially the kinases involved in NRT2.1 regulation are yet unknown. In a screening approach involving phosphoproteome profiling under different nitrogen regimes and by using mutants in transport and/or calcium signaling, we identified several kinases as putative candidate regulators of NRT2.1.

Here we present a leucine-rich-repeat receptor-like kinase H2O2-Induced Ca2+ Increases Like 1 (HPCAL1) as a post-transcriptional regulator of nitrate transporter NRT2.1. HPCAL1 is a close homologue of HPCA1 kinase that functions as a central receptor of extracellular H2O2 for the propagation of the ROS signal during light stress. We propose that, in a similar fashion, HPCAL1 as a possible link between sensing and accumulation of ROS and Ca2+ cascades for cell-to-cell signaling under different nitrate conditions.

Furthermore, with CIPK25 we propose a link of nitrate signaling with the calcium signaling pathway. Studies using Calcineurin B-Like (*cbl5x*) quintuple mutants (*cbl1/4/5/8/9*) revealed a positive correlation between phosphorylation of NRT2.1 at S11 and phosphorylation at the C-terminal regulatory domain of the Calcineurin B-Like Interacting Kinase 25 (CIPK25) T440 site. This suggests a possible CIPK25-NRT2.1 interaction either directly or indirectly.

Elucidation of these interaction partners and pathways will provide new insights into the regulation, sensing, and signalling related to NO3- availability and uptake via the NRT2.1 transporter.

The RGF1-PLT2 regulatory network maintains primary root meristem activity in low phosphate environments.

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Plant root growth rates and patterns exquisitely reflect their above- and below-ground environment and cues from intrinsic physiology and metabolism. Despite much progress in understanding signals and mechanisms underpinning meristem maintenance, how physiology and metabolism modulate meristem activity remains not well understood mechanistically. Recent work by several groups has identified the auxin-RGF1-PLT2 regulatory network as central to the dynamic control of root meristems and hence, root architectural elaboration. We find that this network plays a key role in balancing the distribution of growth activity, and hence root architecture, in conditions of significant phosphate limitation. By regulating the abundance of PLT2 in the primary meristem mediated by the sulfation of RGF peptides, tyrosyl protein sulfo-transferase (TPST) controls root system architecture. We find that metabolic and physiologic state controls TPST's ability to sulfate RGF peptides. Thus, by maintaining stem and transient-amplifying cell homeostasis, the RGF1-PLT2 regulatory network is at the nexus of growth and metabolic control. By underpinning primary root sink strength, TPST orchestrates the distribution of growth activity across the root system and mediates growth responsiveness to a key limiting nutrient, phosphate.

Deciphering the mechanism for brassinosteroid receptor kinase protein BRL3 in plant adaption to drought stress

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Brassinosteroids (BRs) are essential regulators of plant growth and development that are necessary for plant adaption to environmental changes. They are perceived at the plasma membrane by the receptors BRI1 (BRASSINOSTEROID INSENSITIVE 1) leucine-rich repeat receptor-like kinase (LRR-RLK) family. The former receptor BRI1 is ubiquitously expressed promoting growth in basal conditions whereas the vascular BRI1-like (BRL) receptor BRL3 modulates plant adaption to drought and elevated temperatures. In this study, we aim to characterize the central elements of the BRL3 receptor signalosome that account for plant adaption to abiotic stress. Biochemical analysis of BRL3 interactome identified the chaperone ERD14 (Early Response to Dehydration 14) as a BRL3 interactor candidate via mass spectrometry analysis. Here, we will present genetics, cell biology and biochemistry to demonstrate that ERD14 modulates BRL3 levels at the membrane and is essential to confer plant adaptation to several abiotic stresses mediated by BRL3.

Functional study of CLE peptides regulating arbuscular mycorrhizal symbiosis

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CLAVATA3/ESR (CLE) peptides are small signaling hormones playing a key role in plant physiology, regulating diverse processes ranging from cell cycle to symbiotic interactions. CLE peptides were initially considered to be specific to plants, but bioinformatic studies identified a novel CLE-like peptide (eg.RiCLE1) encoded specifically by arbuscular mycorrhizal fungi (1). Interestingly, the exogenous application of RiCLE1 promotes fungal colonization. RiCLE1 sequence is highly similar to three yet uncharacterized *Medicago truncatula* CLE peptides (MtCLE16, 44 and 52), predicted to regulate meristematic activity. Moreover, *MtCLE16* and *MtCLE52* are induced in symbiotic conditions. We used a reporter gene system and promoter deletions to investigate gene expression and identify cis-regulatory regions driving *MtCLE16* expression pattern. We discovered that *MtCLE16* and *MtCLE52* exhibit a symbiosis-specific expression pattern, and we identified a 400 bp cis-regulatory region responsible for *MtCLE16* symbiotic expression. Ongoing multi CRISPR editing and transcriptomics will further elucidate the molecular targets and symbiotic role of CLE peptides. Establishing AM symbiosis involves cell division, differentiation and endoreduplication events necessary for intracellular symbiont accommodation. Therefore, we hypothesize that the fungal CLE-like mimics and novel plant CLE peptides could modulate cell identity related mechanisms in order to facilitate fungal internal accommodation.

(1) Le Marquer et al., 2019 *New Phytologist*

Genetic and functional diversity of Lotus malectin-like domain leucine-rich repeat receptor kinases in root endosymbiosis

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Arbuscular Mycorrhiza (AM) and Root Nodule Symbiosis (RNS) involve a sequential epidermal and cortical signalling modules to establish intracellular accommodation of the microbial symbiont. Two receptor kinase families are required for epidermal infections, including the malectin-like domain leucine rich repeat receptor kinase Symbiosis Receptor Kinase (SymRK) (1, 2, 3, 4). While SymRK is indispensable for epidermal infection in AM and RNS, we tested whether it is also required in the cortex. We observed that tissue-specific expression of *SymRK* in the root epidermis was sufficient to restore AM and RNS in a *symrk* mutant. Based on this observation, we hypothesized that SymRK-like receptors could be involved in cortical signalling in root endosymbiosis. To explore this hypothesis, we identified a family of SymRK Homologous Receptor-Like Kinases (SHRKs) (5) and investigated their roles in root endosymbiosis. In a preliminary analysis of root endosymbiosis phenotypes, *shrk LORE1* insertion mutants displayed phenotypes different from *symrk*. In addition, *SHRKs* clustered into two potentially different functional groups based on their phylogenetic relatedness and the observed phenotypes. In this work, we aim to dissect the evolutionary, functional and molecular relationship between SymRK and SHRKs.

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Signaling peptides regulating root endosymbioses in the model legume Medicago truncatula

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Plants have a high plasticity to adapt to environmental constraints and to optimize their nutrition, notably in their root system. Under limiting Phosphorus (P) and/or Nitrogen (N) conditions, legume plants form symbioses with two types of microorganisms: atmospheric N-fixing symbiotic bacteria, called rhizobia, and arbuscular mycorrhiza fungi. During symbiotic nodulation, a new root-derived organ is produced to house N-fixing bacteria, whereas during mycorrhization, specialized structures for nutrient exchanges, called arbuscules, are formed within root cortical cells. The establishment of these symbioses comes with a high energy cost for the plant, and thus need to be tightly regulated depending on the plant nutritional P and N needs. This is achieved thanks to various local and systemic (ie long distance) pathways notably involving plant signaling peptides. In this project, we selected three families of related signaling peptides suspected to be involved in the regulation of these symbiotic interactions: CEP (C-TERMINALLY ENCODED PEPTIDE), CLE (CLAVATA3/ESR related) from the TDIF (TRACHEARY DIFFERENTIATION INHIBITORY FACTOR) subfamily, and IDA (INFLORESCENCE DEFICIENT IN ABSCISSION) peptides. Progress on these different topics will be reported.

Towards an atlas of REMORIN-nanodomains associated functions.

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Plasma membrane lipids and proteins are dynamically organized into diverse nano-environment giving rise to nanodomains (NDs). The dynamic regulation of plasma membrane organization into nanodomains has recently emerged as a predominant aspect of cellular signaling in plants, but the molecular events underlying context-dependent membrane re-organization and their functional consequences remain largely unknown. REMORINs are structural component of the plasma membrane which tend to be organized in diverse NDs and are proposed to host specific signaling pathways. However, the molecular functions associated with REMs nanodomains are obscure. To answer this question, we are performing an organism-wide functional characterization of REMORINs nanodomains architecture, composition, and function. Our study suggests that as part of *Arabidopsis thaliana* root developmental program, plasma membrane nano-environments of diverse nature are generated in different cell-types to specialize cellular function and to coordinate specific signaling pathways. Through the meta-analysis of proteomics and single-cell transcriptomics data we identified a putative signaling pathway regulating pH and root developmental transition. Our project will shed light on REMORIN-mediated regulation of cell surface signaling across different cell types and tissues and their coordination.

Balancing cell proliferation and differentiation in the vascular cambium

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The vascular cambium is a somewhat unusual stem cell niche in that it is bifacial in nature. Xylem cells are derived from precursors on one side of the cambium, while phloem cells form on the opposing side. This arrangement makes the cambium an interesting model for studying developmental biology. Vascular expansion requires tight regulation of rates of cell division within the cambium, and differentiation at its edges to maintain tissue organisation. Cell division and differentiation are under the control of non-cell autonomous signalling. TDIF, a peptide ligand, is expressed in the phloem, and signals to PXY, a receptor kinase expressed in the xylem-adjacent cambium. Active TDIF-PXY complexes activate transcription factors that promote cell division. Stem cell niches are generally characterised by opposing molecular mechanisms that balance cell division with differentiation, but factors that oppose PXY-signalling have not been described. Here, we show that a set of transcription factors, members of the PLINC family, repress vascular cell division. We discuss a putative mechanism for how the PLINC factors may perform this function. Evidence suggests that PLINC transcription factors interact with the promoters of TDIF-PXY targets, thus, we hypothesise that they act as transcriptional repressors of these cell division-promoting factors. Such a mechanism could contribute to maintenance of the balance between cell proliferation and differentiation in the vascular cambium.

A self-regulatory receptor module involved in edge-based control of plant growth

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The plant-specific GTPase RAB-A5c defines an endomembrane trafficking pathway targeted at cell geometric edges in growing tissues of Arabidopsis (Kirchhelle et al., 2016 *Dev Cell*). Inhibition of RAB-A5c function produces severe defects in unidirectional growth and cell geometries, whilst in silico work has identified cell edges as likely hotspots of mechanical stress during growth. Plant cell geometric edges are thus emerging as likely important but underappreciated cellular domains in control of plant morphogenesis. However, the mechanism by which edge-localised RAB-A5c acts on cell growth remains unknown, and no cargoes of this edge-directed trafficking pathway had been identified. Using a proteomics approach, we have now identified and validated two Receptor-Like Proteins, RLP4s, as components of edge-directed trafficking in growing tissues of Arabidopsis. Our data indicates that RLP4s are trafficked to the plasma membrane in a RAB-A5c-dependent manner, and that they are edge-restricted within the plasma membrane itself, thus occupying a previously unknown polar domain within the Arabidopsis plasma membrane. Using a series of protein truncations, we show that RLP4s associate with the cell wall via their extracellular domains, and require this association for their stability at the cell surface. We have also used mutant analyses and conditional over-expression of RLP4s truncated variants to show that these receptors function in control of cell geometries and unidirectional growth during Arabidopsis development. Finally, we present evidence that RLP4s, as well as being cargoes of RAB-A5c-mediated edge-directed trafficking, function simultaneous upstream of RAB-A5c by promoting its localisation to cell edges. Taken together, our results suggest that RLP4s are components of a self-regulatory growth-control system centred around cell geometric edges in growing tissues. Through this, we provide insights into the molecular mechanisms underpinning how plant cells may sense and alleviate spatially-restricted cell wall perturbations that occur during growth.

Molecular basis for differential CIF peptide sensing by the receptor kinase GSO1 and GSO2/SGN3

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Integrity signaling in the embryonic cuticle requires the leucine-rich repeat receptor kinases GASSHO1 (GSO1) and GSO2 and their cognate peptide ligand TWISTED SEED1 (TWS1). A similar signaling pathway monitors Casparian strip formation in the root endodermis, but depends only on GSO1/SGN3 and the CASPARIAN STRIP INTEGRITY FACTORS CIF1 and CIF2, which share only moderate sequence homology with TWS1. We have previously reported the structure of GSO1/SGN3 bound to CIF2 and de ned a larger family of CIF peptides, part of which are additionally involved in pollen wall formation. How these sequence-diverse CIF peptides can be selectively sensed by GSO1 and GSO2 is unknown. We have now determined high-resolution cryo-electron microscopy structures of GSO1 and GSO2 in complex with different CIF peptides. Combining these structural data with quantitative CIF binding assays, we define how GSO1 and GSO2 have evolved to perform partially overlapping and partially unique signaling functions in plant development. Structures of GSO1 and GSO2 in complex with CIF3 and SERK1 suggest a conserved activation mechanism for both receptors and highlight the contribution of the co-receptor kinase to CIF peptide sensing. Together, our work highlights the structural plasticity of some plant peptide receptor kinases, enabling them to fulfill diverse signaling functions in plant development and immunity.

Uncovering the role of PBLs in signalling specificity in the endodermis

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Plants sense and integrate different environmental inputs, and maintain their information during signal transduction so that diverse cellular responses can be elicited. The mechanisms underlying signalling specificity are badly understood and have so far only been studied in whole plants, entire organs, or artificial systems like protoplasts.

The endodermis is a cell layer that plays an essential role in establishing the main extracellular diffusion barrier.

We use Arabidopsis root endodermis as an *in planta* cellular model system to analyze signalling speci city at a single-cell level and in a native developmental context, using two well-studied plant signal perception pathways: FLS2 and SGN3. Upon the signal-peptide perception, SGN3 and FLS2 associate with and activate type VII receptor-like cytoplasmic kinases (RLCK VII, also known as PBLs) SGN1 and BIK1, respectively. PBLs are a large class of proteins involved in the early stages of signal detection and are well-suited candidates to be critical nodes for determining cellular signaling specificity.

Here, we demonstrate that FLS2 signaling in the endodermis does not require the highly abundant SGN1 PBL kinase, which is strongly needed for SGN3 signaling but instead requires BIK1, despite its low expression in the endodermis. This suggests specific requirements for different PBL signaling interactions. We are currently conducting CRISPR knock-outs of all endodermally-expressed PBL members in order to identify the complete set of PBLs required for SGN3 or FLS2 signaling in the endodermis. Based on this, we aim to understand the molecular basis of LRR-RLK signaling specificity at a single-cell type level.

The SCHENGEN Pathway Orchestrates the Protective Barrier of the Periderm

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Apoplastic barriers play a vital role in plant homeostasis, growth, and development. The proper function of these barriers is monitored by surveillance mechanisms that ensure plant protection. Until now, the only well-documented example is the SCHENGEN pathway. It regulates and monitors Casparian strip formation, guarantees the proper embryonic cuticle formation during seed development and, orchestrates modifications of pollen wall components within the anther locule. The activation of this pathway involves the perception of peptide ligands released into the plant apoplast by leucine-rich repeat receptor-like kinases (LRR-RLKs) and a cytoplasmic kinase specifically localized. Altogether this leads to the proper deposition of suberin, lignin, or cutin, depending on the developmental contexts. However, if a similar surveillance system is active in other barriers, such as the periderm, is unknown. In the periderm, the armor that protects the plants during radial growth, the regulation of the suberin and lignin deposition is still poorly understood. To shed light on these processes, we investigate whether the SCHENGEN pathway plays a role in periderm differentiation. By employing fluorescent reporters, we found that many components of the Schengen pathway are expressed in distinct tissues of the periderm. Moreover, the Arabidopsis periderm can respond to the exogenous application of SCHENGEN peptide ligands by activating suberin and lignin deposition. In addition, the analysis of loss of function mutants suggests that the Schengen pathway regulates periderm barrier establishment. Finally, the understanding of how the SCHENGEN pathway regulates the establishment of the periderm barrier will provide new insight into how to engineer the periderm barrier to render plants more salient to stresses.

Regulation of maize ear development by a CLAVATA-related receptor complex

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Plant organ size and shape is dictated by the control of meristem proliferation and differentiation. Meristem size is controlled by the CLAVATA (CLV)-WUSCHEL (WUS) signaling pathway, which involves an interplay between CLV receptors, their ligands, and the mobile transcription factor WUS. Maize mutants lacking CLV receptors have fasciated ears, with flattened tips and disordered kernel rows, a result of overproliferating meristematic cells. One such mutant, *fasciated ear3 (fea3)*, lacks a functional copy of a leucine rich receptor-like protein. Weak *fea3* alleles increase ear size without a compensatory loss in seed size, making this an attractive target for yield enhancement. The molecular mechanism by which FEA3 exerts its control on meristem size is unknown, as FEA3 is expressed in a spatially distinct domain from other *CLV* receptors, and *fea3* interacts additively with other *CLV* receptor mutants. Intriguingly, a *CLV1* paralog, *ZmBARELY ANY MERISTEM 1D (ZmBAM1D)*, is upregulated in *fea3* mutants. *FEA3* and *ZmBAM1D* expression overlaps in the center of spikelet meristems and the two proteins interact when co-expressed in *N. benthamiana*. While *bam1d* does not have an ear phenotype, *fea3;bam1d* mutants are more fasciated than *fea3* mutants, suggesting that the two genes interact. Furthermore, both FEA3 and ZmBAM1D perceive the same CLE peptide ligand. These observations suggest that FEA3 and ZmBAM1D may form a receptor-co-receptor pair. We are validating the interaction between ZmBAM1D and FEA3 and discovering additional *in vivo* interactors using proximity labeling with TurboID, which can better resolve transient protein-protein interactions compared to immunoprecipitation-based approaches. Since BAM receptors have pleiotropic roles in plant development and defense, comparing the proximity labeling interactome of ZmBAM1D and FEA3 may also help reveal how signaling specificity is achieved in different biological contexts. With a deeper understanding of how these receptors regulate meristem activity, we can more precisely engineer this process to enhance yield-related traits.

Mutual regulation of canalization by CLE peptides and auxin in Arabidopsis

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Auxin canalization is a fascinating process that is crucial for important developmental decisions, such as those involved in organogenesis, embryogenesis, vascular tissue formation, and regeneration. Auxin canalization hypothesis-predicted behavior is best observed during *de novo* formation vasculature, especially of new regenerated vessels after incision, i.e. wounding or grafting. A key role of auxin in promotion of canalized flow by itself and transport channels formation is well accepted. Ultimately, canalized auxin flux provides positional information guiding the paths of new vasculature. The impact of auxin on the polar PIN1 positioning might be part of a mechanism for canalization, possibly occurring via coordinated auxin effects on (i) PIN expression via transcriptional TIR1/AFBs pathway and at the same time on (ii) PIN polarity via extracellular auxin signaling. The underlying mechanism is so far elusive and the knowledge on molecular players is very limited. Some recent discoveries found that plant receptor-like kinases, Canalization-related Auxin-regulated Malectin-type RLK (CAMEL), and Canalization-related Receptor-like kinase (CANAR), as well as TRANSMEMBRANE RECEPTOR-LIKE KINASEs (TMKs) and its associated Auxin Binding Protein 1 (ABP1), are important players in this process. Here, we found that a group of CLE peptides, which are auxin-induced and in turn negatively regulate auxin distribution in root, are essential for auxin feedback on its transport and for canalization-mediated processes. They show antagonistic function in *de novo* auxin channels formation and auxin polar transporting, meanwhile, their receptors interact with LRR-RLKs TMK1/4 for further association with PIN1 on the cell membrane to regulate its trafficking and repolarization during canalization. Thus, our study provides the mechanistic insights of CLE peptides and auxin mutual regulation in canalization.

Role of hormone peptides in the systemic regulation of white lupin cluster roots development

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To understand the mechanisms that allow plants to use phosphate more efficiently and grow in nutrient-poor soils, we need to increase our interest in plants that are genetically adapted to these nutrient deficiencies. White lupin can be cultivated with limited addition of phosphate fertilizers due to the production of spectacular structures, made of hundreds of closely spaced and highly specialized lateral roots, called cluster roots. Using a forward genetic approach we have selected several white lupin mutants *constitutively producing cluster roots (ccr)* and identified key genes involved in cluster roots development. LaCCR1, that was identified as a receptor-like kinase belonging to CLAVATA1 family, has been shown by grafting experiments to be a systemic inhibitor of cluster roots development, while LaCCR2, whose identification is still in progress, locally controls the development of this organ. Plant hormone peptides are known to play crucial roles in the long-distance signalling pathways that regulate plant development and response to environmental factors. By using a peptidomic approach we successfully identified 11 post-translationally modified peptides belonging to the peptide family CEP (C-terminally encoded peptides) in white lupin xylem sap. The quantification of the identified CEP peptides by LC-MS/MS in the wild type and *ccr* mutant backgrounds demonstrated the up-regulation of peptide translocation in ccr1 mutant xylem sap. The over-accumulation of several CEP peptides in ccr1 mutant suggests CEP signals being feedback-regulated by the receptor LaCCR1. CEP transcripts were shown to be up-regulated in the roots of both *ccr1* and *ccr2* mutants. However, CEP peptides root-to-shoot translocation was downregulated in *ccr2* mutant, thus suggesting that LaCCR2 might be implicated in hormone peptides maturation in roots. In-depth study of hormone peptide dynamics in *ccr* mutants will allow us to better understand how long-distance regulations control the development of cluster roots.

Dynamic remodeling of the anionic lipid landscape by RALF peptides

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Anionic phospholipids play pivotal roles in plant development and environmental responses. Despite their low abundance, these lipids serve as crucial regulators by recruiting a diverse array of proteins, influencing processes such as growth, cell division, cell trafficking, and cellular signaling. On the other hand, FERONIA, a plasma membrane receptor kinase, participates in numerous biological processes and can be activated by peptides from the RALF family. Most RALF, including RALF23 and RALF1, are implicated in cell wall alkalinization and root elongation inhibition, yet they also trigger isoform-specific output.

We recently demonstrated that RALF23 impacts Rho GTPase signaling by controlling the subcellular localization of phosphatidylserine, a membrane anionic phospholipid. This effect is both FERONIA-dependent and highly dynamic. Indeed, phosphatidylserine translocates from endosomes to the plasma membrane in a reversible manner upon RALF23 treatment.

Here, we quantitatively describe this complex intracellular dynamic by coupling high spatiotemporal resolution confocal microscopy with microfluidics. By following through time, the subcellular localization of fluorescent phosphatidylserine reporters, we confirmed a biphasic response upon RALF23 treatment. Furthermore, we detected an enrichment of the phosphatidylserine probe at the cell surface within minutes following RALF23 treatment. We next addressed the impacts of RALF23 on the localization of other anionic lipids. We found that RALF23 also regulates the localization of phosphatidylinositol 4-phosphate (PI4P). Intriguingly, RALF23 promotes the endosomal localization of PI4P indicating that it has the opposite effect on phosphatidylserine and PI4P lipid localization. Furthermore, we found different lipid responses when using RALF1 instead of RALF23. Together, our data highlights the deep and rapid impact of RALF peptides on anionic lipids dynamics within plant membranes. It also illustrates how different RALF peptides may use distinct lipid second messengers to execute specific signaling programs.

Optogenetic approach to study stem cell fate regulation via Calcium-fluxes within the root of Arabidopsis thaliana

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Plants are multicellular organisms where many different specialized cell types must communicate with each other and their biotic environment to create a functional unity. Therefore, channels serve as tools for nutrient exchange and communication between cells. Especially stem cells (SC) that need to undergo cell fate decisions must tightly communicate with their surroundings. The underlying mechanisms are not yet fully understood. Major players in communication within SC-containing tissues, so called meristems, are secreted peptides, including CLAVATA3/EMBRYO SURROUNDING REGION40 (CLE40). CLE40 is known to control the differentiation status of meristematic root cells. It was hypothesised recently by Breiden *et al.* that ion fluxes, here Ca2+, are involved in cell fate decisions of *Arabidopsis thaliana* root SCs, but the precise mechanism and relation to CLE40 remain unidentified. Root SCs are essential for root growth and development. The control of their maintenance and number requires a robust regulatory network and is driven non-cell-autonomously by a small group of cells in their centre, the quiescent centre. Non-cell-autonomous signal transduction is essential for the communication between cells and can, among others, be realized by ion fluxes through channel proteins. A non-invasive manipulation of such ion fluxes could help understanding their function during plant development, including cell fate decisions within meristematic tissues. Optogenetics as a light-sensitive instrument to control cellular activities has greatly advanced neuroscience but was not applicable in plants because of poor expression levels and a missing cofactor, until recently. Zhou *et al.* developed an efficient optogenetic tool for *in planta* usage based on a light-sensitive channel-rhodopsin and thereby paved the way for detailed *in planta* analyses of complex regulatory networks including outputs of channel fluxes. In my work I want to elucidate how changes in Ca2+ concentration of meristematic root cells could drive SC fate decisions and if this depends on CLE40 signalling. I will realise this with a combination of genetic and biochemical approaches, using advanced fluorescence microscopy as a major tool. I will link *in planta* optogenetics to fluorescent Ca2+-sensors such as R-Geco. This allows to uncouple the Ca2+ release from CLE40 signalling and results in a light-inducible and trackable Ca2+-flux within specific meristematic cells, ready to characterize the subsequent cell fate decisions.

P-1
To die or not to die? Role of lectin receptor kinases in extracellular ATP-mediated regulated cell death in Arabidopsis

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Plant regulated cell death (RCD) is a tightly controlled process involved in various developmental and stress responses. Upon pathogen perception or heat stress, for instance, RCD allows removal of damaged cells, thereby contributing to adaptation. The extracellular nucleotide adenosine triphosphate (eATP) has emerged as a signaling molecule that participates in RCD in several plant species, including Arabidopsis. Indeed, published data suggests that both increased and decreased levels of eATP can lead to RCD. In Arabidopsis, eATP is perceived by the purinoreceptors P2K1 and P2K2, which belong to the lectin receptor kinase family. The Arabidopsis *p2k1/p2k2* double mutant is compromised in eATP perception and signaling. However, we have limited knowledge on the role of P2K1 and P2K2 in RCD triggered by eATP. To investigate the role of P2K1 and P2K2 on eATP-mediated RCD in Arabidopsis we subjected Col WT and *p2k1/p2k2* double knockout plants to exogenous ATP treatments and assessed RCD. Cell death was quantified by evaluating electrolyte leakage, as RCD leads to the loss of plasma membrane integrity and subsequent ion leakage from cells. Additionally, Trypan Blue staining was performed to assess cell viability. Preliminary results suggest Col WT plants exhibited enhanced electrolyte leakage over time compared to the buffer-treated control, indicating ATP-induced RCD. In contrast, the *p2k1/p2k2* plants displayed reduced electrolyte leakage, suggesting eATP-induced RCD is mediated by the purinoreceptors P2K1 and P2K2. The differential RCD observed in Col WT and *p2k1/p2k2* plants emphasize the significance of purinoreceptors in modulating the sensitivity and intensity of eATP-induced RCD. Further investigation into the downstream signaling components and molecular pathways associated with purinoreceptors will deepen our understanding of plant RCD control.

P-3
The mechanism of PEP7 maturation in SIRK1 signaling pathway

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Plant receptors constitute a large protein family that regulates various aspects of development and responses to external cues. Functional characterization of this protein family and the identification of their ligands remain major challenges in plant biology. Previously, we identified plasma membrane-intrinsic sucrose-induced receptor kinase 1 (SIRK1) and Qian Shou kinase 1 (QSK1) as receptor/co-receptor pair involved in the regulation of aquaporins in response to osmotic conditions induced by sucrose. Recently, we identified a member of the elicitor peptide (PEP) family, namely PEP7, as the ligand of SIRK1, which binds to the extracellular domain of SIRK1 with a binding constant of 1.44 ± 0.79 mM, stabilizing the SIRK1-QSK1 signaling complex. *In vivo* phosphorylation of aquaporin and water in flux into protoplast was induced upon PEP7 treatment. However, a systematic inquiry of the link between sucrose stimulation and PEP7 appearance has not been established so far. In this study, we proposed preliminary results regarding the effect of sucrose on PEP7 generation and PROPEP7 post translational modification. We hypothesis that the regulation mechanism of the post translational modification of PROPEP7 may play a key role in sucrose regulated PEP7-SIRK1 signaling pathway.

P-4

ROS-dependent extracellular interaction network of Cysteine-rich and Leucine-Rich Repeat Receptor Kinases in Arabidopsis

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In plants, like other multicellular organisms, extracellularly produced Reactive Oxygen Species (ROS) are crucial components of multiple signalling pathways for communication between the environment and the interior of the cell. Sessile plants are constantly exposed to ever-changing environmental conditions and ubiquitously use ROS as signalling molecules in many biological processes, including stress responses and development. It, however, remains largely unexplored how extracellular ROS are sensed and how they modulate diverse cellular responses. Cells use a variety of sensors or receptors to monitor their environment. A critical class of such sensors are the Receptor Kinases (RKs), which detect both 'self' and 'non-self' derived signals to trigger appropriate cellular responses. To understand how extracellular sensing machineries of ROS and RKs are interconnected, we created system-wide interaction maps between extracellular domains of the two largest families of RKs, Leucine-Rich Repeat Receptor Kinases (LRR-RKs) and Cysteine-rich Receptor Kinases (CRKs), in the presence and absence of ROS. The resulting high-confidence interactions were plotted in an interaction map and further filtered according to their co-expression in different tissues and developmental stages. This dataset represents a valuable source of information and insight for further research on the effect of ROS signalling on the biochemical dynamics of extracellular receptors.

P-6
An endogenous peptide signal in Arabidopsis modulates components of the Iron deficiency response.

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Iron (Fe) is an essential element for most of the living organisms and plants are the primary source of dietary iron to humans. Thus the understanding of iron uptake, acquisition and utilization becomes crucial in plants. There are several studies that provide ample evidences for role of phytohormones in regulating iron homeostasis. But peptide signaling-mediated Fe homeostasis is less explored. In this work, we aim to study the role of PEPR (Perception of *Arabidopsis* danger signal peptide receptors) and their ligands i.e. PEPs (Plant Elicitor Peptides) in plant growth and development under Fe deficiency stress condition. We find that *AtPROPEP2* expression is significantly induced under Fe deficiency. The *pep2* transgenic plants exhibited increased tolerance to Fe deficiency stress conditions and accumulated more Fe as compared to WT plants. Additionally, we have also found that rhizosphere acidification in response to Fe deficiency is strongly affected in *pep2* plants. Collectively, these data indicate that AtPep2 plays a crucial role in Fe deficiency signalling pathway in plants.

P-7
Pursuing PSY1R and PSY peptides – unravelling the dynamics of a LRR-RK signaling pathway

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Acidification of the cell wall space outside the plasma membrane is required for plant growth. This acidification is the result of proton extrusion by plasma membrane-localized H+-ATPases. In Arabidopsis thaliana, the leucine rich repeat receptor kinase (LRR-RK) PSY1R directly phosphorylates and activates the plasma membrane H+-ATPase, AHA2. Application of plant peptides containing sulphated tyrosine (PSYs) to seedlings has shown to increase proton pumping in roots. This activity was not observed in *psy1r* mutant seedlings (Fuglsang et al. 2014). The PSY peptide family consists of nine homologs (PSY1-9). All nine PSYs share a highly conserved domain at the c-terminus. Despite their highly conserved active domain, the nine homologs do not all share the same post-translational modifications. Recent studies suggest that the PSY homologs are perceived by different receptors, serving different physiological functions (Ogawa-Ohnishi et al. 2022). The roles and functions of the PSY peptides have shown difficult to identify as the nine homologs have redundant functions, though their expression pattern is tissue- and even cell specific for some homologs (Tost et al. 2021). To understand the specific modes of action of PSY peptides a fluorescence energy transfer (FRET)-based receptor system will be developed for live imaging of active PSY peptides in plants. Using this system, receptor-peptide binding kinetics and the specificity of single PSY homologs will be investigated.

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P-8

Molecular insight into cell wall integrity signaling in Arabidopsis mediated by the receptor kinase STRUBBELIG

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Plant cells are uniquely characterized by a biochemically complex, semi-rigid cell wall. Most molecular pathways occurring in the cell are directly or indirectly influenced by the cell wall, including yield-related processes. In turn, the physicochemical properties of the cell wall are modulated by growth signals and abiotic and biotic stresses. In recent years, there has been increasing evidence that plant cells actively monitor cell wall integrity and initiate complex compensatory responses to changes in cell wall properties that occur during growth or in the presence of abiotic and biotic stresses. However, our molecular understanding of the intricate communication mechanisms at the plasma membrane-cell wall interface is fragmentary at best (1). We recently demonstrated that the receptor kinase STRUBBELIG (SUB), previously known for its essential function in tissue morphogenesis, plays an important role in cell wall remodeling following cell wall damage induced by cellulose biosynthesis inhibition (CBI) (2). Here, we address the composition of SUB-containing cell surface receptor complexes. We will present results from co-immunoprecipitation experiments using a functional SUB:EGFP reporter as bait. We have identified several cell surface signaling factors as candidate components of SUB receptor complexes. In addition, we genetically verified a role in the CBI response for a subset of these factors. We will present our progress in understanding the molecular architecture of SUB receptor complexes mediating cell wall remodeling in response to cellulose biosynthesis defects.

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P-9
The role of RLKs-RLCKs complexes in low potassium signaling

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Receptor-like kinases (RLKs) and receptor-like cytoplasmic kinases (RLCKs) complexes function in the perception of many environmental cues and crucially regulate various developmental processes. However, their potential contribution to sensing and adaptation to limited supply of the essential macronutrient potassium (K+) is only fragmentary characterized. We recently reported that CIF1/2 peptides activate GSO1/SNG3-LKS4/SGN1 modules in response to Low K+ (LK) in order to facilitate accelerated formation of casparian strip and transcriptional induction of the high affinity K+ uptake transporter HAK5. Moreover, it was reported that the co-receptor kinase BAK1 contributes to LK tolerance. However, it remained unknown which RLK functions with BAK1 in LK responses and if and how other RLKs potentially contribute to LK signaling. We performed data base searches to identify candidate RLKs which expression is modulated in response to LK exposure (LK-induced Receptor Kinases; LIRKs). Here we will present the results of our phenotypical characterization of loss of function mutants of LIRK1 and LIRK2, which both display discernable phenotypes in various LK assays compared to wild type. We will also discuss our strategy to identify the respective peptides that convey LIRK activation.

P-10
Mapping the ligand binding spectrum of the brassinosteroid receptor kinase family from Arabidopsis

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Brassinosteroids (BRs) are a class of growth-promoting steroid hormones. Arabidopsis contains a complex network of parallel biosynthetic pathways for BRs. Currently, brassinolide is considered the final and most bioactive BR. BR perception and signaling is mediated by a small family of receptor kinases composed of four receptors: BRI1, BRL1, BRL2 and BRL3 and their cognate SERK co-receptors. We have combined grating coupling interferometry (GCI) and Xray crystallography to perform a structure – activity analysis of the different BR receptors in Arabidopsis. We find that BRI1 and BRL1/3 can sense a wide range of different BRs present in Arabidopsis with high affinity. Structural and mutational analysis of BRI1/BRL3 complexes bound to different BRs de ne the main chemical features required for bioactivity. We correlate these in vitro data with BR metabolomics from wild-type Arabidopsis plants, and from BR biosynthetic and signaling mutants. Structure-guided design of novel BR antagonists inhibit the interaction of BRI1 and BRL1/3 with their SERK co-receptor kinases. A structure of BRL2 reveals why this sequence-related receptor cannot sense BRs. Together, our work defines BRI1/BRL1/BLR3 as biochemical equivalent broad spectrum BR receptors that use a conserved activation mechanism.

P-11
Uncovering the role of CLE signalling in xylem differentiation

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CLE peptides are unique land plant-specific signaling peptides that control meristem activity, phloem development, and interaction of the plant with other organisms and the environment. In Arabidopsis, 33 genes encode for such peptides, which are transcribed, translated, processed, and secreted to the apoplast as active peptides. In my project, I focus on xylem-specific CLE peptides and aim to better understand their biological role in xylem formation. To identify xylem-expressed *CLE* genes I created fluorescent reporter lines of all *CLE* genes in Arabidopsis. Using these in combination with single-cell transcriptomics on Arabidopsis roots I was able to find expression in xylem of 6 *CLE* genes. Using targeted mutagenesis, I was able to target all 6 genes to create higher order mutants. Microscopic analysis of these mutants paired with tissue-specific inducible expression of *CLE* genes pinpointed their role in promoting xylem lignification onset. To discover molecular components in this pathway I took a proteomics approach. LC-MS/MS analysis of the CLE receptor BAM1 helped identify a novel Receptor-Like Cytoplasmic Kinase that specifically interacts with CLE receptors. Phylogenetic analysis of this novel interactor identified several closely related homologs, all of which I am targeting for mutation. I will present my latest results on this previously uncharacterized RLCK and additional interactor that play a key role in the transduction of the CLE signals in relation to xylem development.

P-12
Hechtian attachment sites – connecting the plasma membrane to the cell wall

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In all multicellular organisms, cells are surrounded by a meshwork of polymers that is deposited by the cells, signals back to the cells, and is remodelled by them in a feedback loop. In animals, the meshwork forms a soft extracellular matrix (ECM) and the attachment of plasma membrane to ECM contributes to both ECM deposition and (mechano)sensing. In plants, cells are surrounded by stiff, load-bearing cell walls and the role of plasma membrane – cell wall attachment sites is ill-described. In this project, we analyse Hechtian attachment sites (HATSs) that anchor the plasma membrane to the cell wall at discrete points even in face of plasmolysis. HATSs thus harbour a great potential as mechanosensing hubs, points accumulating both mechanical forces, and mechanosensitive components at discrete points in the cell. We revisit HATSs, aiming to understand their dynamics, their composition, and their role in mechanosensing, as well as the feedback loop in which HATSs contribute to sensing of the mechanical status of plant tissue and modulation thereof.

P-13
BSKs: A convergence point for RLK signaling transduction in plants

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Plant cells continuously survey their environment to ensure a coordinated response to endogenous and exogenous cues. Plants have evolved many receptor-like kinases (RLKs) subfamilies to sense and respond to hormones, developmental peptide regulators, or pathogenic cues, among others. Despite the large array of plasma membrane RLK receptors and the diversity in their extracellular sensing moieties, plant cells appear to utilize a rather reductive set of intracellular effectors to convey the signals. We have recently identified members of the BRASSINOSTEROID SIGNALING KINASE (BSK) family as a redundant set of effectors in the RLK-mediated signaling cascade driving floral organ abscission. Given the involvement of BSKs in various RLK signaling pathways, we have started to characterize BSK1 activity determinants in the context of multiple signaling pathways. In this poster, we draw parallels between BSK-regulated RLK signaling cascades, provide evidence for functional conservation, and contextualize these results with the need to maintain signaling specificity.

P-14
Is KRS a ROS sensor?

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In plants as in animals, extracellular Reactive Oxygen Species (ROS) play important roles in regulating extracellular matrix properties, and in both autocrine and paracrine signaling. ROS are thought to act as important signals in plants, but this activity remains poorly understood. ROS signaling involves oxidative modification of intermediary molecules, often through thiol-based modification of target proteins. Cys-rich peptides (CRPs) may be involved in ROS perception. A recently discovered CRP named KERBEROS (KRS) is necessary for the formation of a glycoprotein rich structure called the Embryo Sheath (ES), which is deposited on the surface of the developing embryo by the neighboring endosperm tissues. Consistent with this, KRS is expressed specifically in endosperm cells adjacent to the embryo. Mutating KRS compromises ES formation and the ES-mediated embryo-endosperm separation, impeding embryo growth, and seedling emergence. The KRS Cys-Rich Domain (CRD) alone is sufficient to complement *krs* mutants, whilst mutating four conserved Cys residues in the CRD abolishes protein function. My proposed project is focused on understanding KRS role during seed development and how ROS signaling in the plant cell wall (apoplast) contributes to seed development and viability. I will test the hypothesis that KRS mediates or modulates the transmission of ROS-dependent signals between seed compartments to ensure proper embryo development.

P-15
A mutation in HISN2 suppresses the dominant negative effect of LRX1dE

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Cell and cell wall growth are mutually dependent processes that must be tightly coordinated and controlled. Leucine-rich repeat extensin 1 (LRX1) of *Arabidopsis thaliana* is a cell wall-localized protein that acts as a sensor of cell wall integrity in root hair development. Loss of LRX1 function leads to a defect in root hair development, with deformed root hairs that frequently burst. The extensin domain of LRX1 consists of Ser-Hyp2-n repeats that anchor LRX1 to the cell wall. Expression of LRX1 with a truncated extensin domain (LRX1dE) results in a dominant negative effect, with a root hair phenotype comparable to *lrx1*. To identify genes involved in an LRX1-related process, a suppressor screen was carried out on Arabidopsis expressing LRX1dE and the *hisn2 (Histidine Biosynthesis 2)* mutant was identified as a suppressor of the dominant negative effect mediated by LRX1dE expression. Amino acid levels are sensed by the Target of Rapamycin (TOR) pathway, which controls eukaryotic cell growth by coordinating a wide range of cellular processes such as transcriptional and translational activities, autophagy, mitochondrial activity, and cytoskeleton dynamics. LRX1dE *hisn2* exhibits reduced sensitivity to the TOR kinase inhibitor AZD-8055, indicating a modified activity of the TOR network. These data are consistent with previous results demonstrating the interlinkage between TOR kinase activity and the LRX pathway in cell wall development.

P-16
Proteolytic processing of the sulfated CIF peptide precursors by subtilisin-like proteases for Casparian Strip formation in Arabidopsis

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In multicellular organisms, water and most small molecules including nutrients, toxic substances and signalling agents move freely through extracellular spaces depending on their biochemical characteristics. To control homeostasis of such compounds, multicellular organisms have evolved extracellular barriers between certain tissue layers, such as tight junctions in animal epithelia. Similar diffusion barriers are also generated in plants by the accumulation of hydrophobic chemicals such as lignin, suberin or cutin in plants cell walls, e.g. the Casparian strip (CS) in roots, the embryonic cuticle in seeds, and the peritapetal strip separating the tapetum from outer tissues in developing anthers. Diffusion barrier formation is tightly regulated and the underlying mechanism have been investigated in most detail for the CS. This barrier forms a hydrophobic band around the radial cell walls of the root endodermis. Its integrity is controlled by the CASPARIAN STRIP INTEGRITY FACTOR (CIF) gene family, with CIF1 and CIF2 encoding propeptides that are processed into small secreted peptides. Posttranslational sulfation of tyrosine residues in CIF peptides is required for full bioactivity and high-affinity binding to the SCHENGEN3/GASSHO1 (SGN3/GSO1) receptor to trigger localized production of reactive oxygen species for spatially controlled lignification of endodermal cell walls. However, it is still unknown how and where the peptide precursors are processed to release the mature peptides. Using tissue-specific promotors driving the expression of subtilase inhibitors, we showed the involvement of subtilases localised in the stele and endodermis. We then took a candidate approach selecting subtilases expressed in these tissues according to their expression patterns revealed by promoter-reporter gene analysis and published single cell RNAseq data. Preliminary in-vivo assays confirmed a role for two of the candidates in CS formation. In vitro assays showed that the CIF1 and CIF2 peptide precursors are processed by candidate subtilases and suggested that R/KDY cleavage site recognition may be facilitated by the posttranslational tyrosine sulfation. These data reinforce the role for posttranslational modification, here tyrosine sulfation, in mediating the specificity of processing and peptide maturation and refine the specificity of cleavage by proteases.

P-17
Interactions between Receptor Kinases define Radial Pattern in Arabidopsis

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Receptor kinases are involved in different plant growth and development processes including secondary growth. Understanding the crosstalk between RLK-mediated signalling pathways is an important focus of ongoing vascular development research. Recent discoveries indicated two receptor kinases, *PXY* and *ER*, genetically interact to coordinate vascular proliferation and organisation via inter-tissue signalling in *Arabidopsis*. Nevertheless, the mechanisms of the interaction and whether interactions are underpinned by physical interactions remains unknown. As the domain of ERL gene expression is expanded in *pxy* mutants, the presence of PXL receptors in cells that also express ERf proteins (ER and its paralogues) is increasingly likely. A direct interaction between members of these receptor families is therefore possible. Strong *in vitro* interactions between ER and PXY, and ER and PXL1 proteins were observed in one orientation, and between PXL2 and ERL2 in both directions. Furthermore, in vivo techniques, FRET and Co-IP techniques were used to con rm the physical interactions between PXfs (PXY and its paralogues) and ERfs. Further work is being conducted currently, hoping to highlight these interactions in more detail.

P-18
Stem cell-promoting CLE peptide signaling in the liverwort Marchantia polymorpha

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Indeterminate growth of plant body relies on the activity of apical meristems in which stem cells are maintained at a relatively constant size under the control of local hormonal signals. In the shoot apical meristem of *Arabidopsis thaliana*, a negative feedback loop involving CLAVATA3 (CLV3) peptide hormone and WUSCHEL (WUS) transcription factor plays a key role in the homeostasis of stem cell population. To understand the evolutionary origin of this cell signaling system, we are studying CLE (CLV3/ESR-related) peptide signaling in the liverwort *Marchantia polymorpha*, a non-vascular land plant model.

We found that the single CLV3 ortholog, MpCLE2, promotes the expansion of the stem cell zone and branching from the meristem. This stem cell-promoting activity of MpCLE2 is contrary to the conventional stem cell-limiting CLV3/CLE signaling in Arabidopsis (Hirakawa et al 2020). Since a CLE gene with similar function, AtCLE40, was reported (Schlegel et al. 2021), it is conceivable that stem cell-promoting CLE peptide signaling is an ancestral pathway conserved throughout land plants (Hirakawa 2022). Instead, a stem cell-limiting CLV3 pathway in flowering plants may be a derived condition evolving after gene duplication in the common ancestor of flowering plants.

To identify the downstream targets of MpCLE2 signaling, we conducted RNA-seq analysis using gain-of-function and loss-of-function alleles for MpCLE2 and its receptors (Takahashi et al. bioRxiv). Among several DETFs, we found a NAC domain transcription factor whose expression was greatly reduced by MpCLE2 signaling. Using CRISPR editing and DEX induction system, we show that this gene, namely JINGASA (MpJIN), promotes periclinal division at the periphery of the SCZ thereby limiting the SCZ size. Since MpJIN homologs are conserved between bryophytes and vascular plants, CLE-JIN pathway may be a mechanism shared between the gametophytic and sporophytic shoot apical meristems in land plants.

P-19

Identification of systemic effectors acting downstream of CEP signaling peptides to regulate symbiotic nodulation in response to nitrogen deficit

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Legume plants adapt their root system architecture to environmental conditions by modifying root growth and lateral root number. In the case of a mineral nitrogen deficit, they develop a new organ in response to rhizobium symbiotic bacteria: the nitrogen-fixing nodule. Local and systemic regulatory pathways coordinate root and nodule development depending on nitrogen availability, notably through signalling peptides perceived by Leucine-Rich Repeats Receptor Like Kinases. In the *Medicago truncatula* model legume, C-TERMINALLY ENCODED PEPTIDEs (CEPs) were reported as critical to determine root competence for symbiotic nodulation through the COMPACT ROOT ARCHITECTURE 2 (CRA2) receptor acting in shoots1-4. Effectors acting downstream of this CEP/CRA2 systemic pathway remain to be discovered either in shoots or in roots.

We used transcriptomic analyses to compare systemic responses of wild-type plants *versus cra2* mutants at an early stage after rhizobium inoculation, or in plants experiencing a nitrogen deficit or satiety. This allowed the identification of candidate genes acting downstream of the CEP/CRA2 pathway in shoots or in roots, either encoding transcription factors from different families or an enzyme involved in secondary metabolite production. Progress on the functional analysis of some of these CEP/CRA2 effectors will be reported.

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P-20
Root-knot nematodes produce functional mimics of tyrosine-sulfated plant peptides

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PSY (plant peptides containing sulfated tyrosine)-family peptides promote root growth and cellular expansion and are proposed to suppress plant defenses. In the biotrophic bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*, a PSY-like peptide named RaxX (required for activation of XA21-mediated immunity X) contributes to bacterial virulence. Here, we report the identification of putative PSY-like peptides from plant parasitic root-knot nematodes of the *Meloidogyne incognita* group (MigPSY). Exogeneous application of MigPSY peptide derivatives promotes root growth in Arabidopsis at levels comparable to application of plant PSY or bacterial RaxX. *MigPSY* localizes to the secretory glands of pre-parasitic juvenile nematodes and transcript levels peak during the early stages of infection. Down-regulation of *MigPSY* gene expression reduces root galling and nematode egg production, suggesting that MigPSYs serve as nematode virulence factors. Together, these results indicate that nematodes and bacteria exploit similar sulfated peptides to hijack plant developmental signaling pathways to facilitate successful infection.

P-21

Kratos gives strength: Functional validation and characterization of an extracellular peptide that promotes cell survival

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To facilitate a response to stress stimuli, plants often utilize peptide-based signals that are predominantly cell-to-cell mobile and help in mounting either a defense-based or a growth-based response to adapt to the situation (Hander and Fernández-Fernández et al., 2019). Previously, an extracellular peptide named Kratos that reduced cell death, both as a response to wounding and during xylem development was identified through peptidomics (Escamez, Stael, Vaionen et al., 2019). However, there have been no reports as to how the peptide is generated nor has the mechanism behind how Kratos exerts its cell survival function been understood. Here we aim to functionally characterize and validate the role of Kratos, a peptide with the potential to promote cell survival in terms of development post wound response and regeneration. Exogenous application with Kratos peptide had a positive effect on root length and regeneration, especially when the pH was adjusted to 7.0. Exogenous treatment with the peptide also reduced grafting efficiency thereby suggesting potential reduction in cell death. A significant feature of the protein that generates the mature 20 amino acid Kratos peptide is the presence of large stretches of Gly residues interspersed with a few Lys residues and that sets it apart from other Gly rich proteins in plants. Phylogenetic analysis revealed a paralog in *Arabidopsis*, which we have named Zelus that shows a similar protein sequence and contains the exact same peptide sequence as Kratos, thereby suggesting existence of other such peptides that may have a role in cell survival. We hypothesize that "preKratos" is processed by a protease resulting in Kratos post wounding or development stimuli and has a role in ameliorating cell death by protecting cells and helping in regeneration. We also aim to characterize Zelus and identify if it generates Kratos-like peptides thereby acting as cell survival peptides. Wounding often results in debilitating damage to a cell resulting in cell death, and while existing research has identified peptides that enhance cell death, it has not yet been established whether certain factors exist that can help cell survival or minimize runaway cell death. To our knowledge Kratos is the first identified peptide in plants that helps prevent cell death in both development and wounding contexts. Functional validation and understanding the mechanism of Kratos has the potential to open new avenues in identification of peptide signals that can potentially promote cell survival and help cells to survive damage and perhaps even death.

P-22
After the receptor: How does GSO1/GSO2 activation control embryo surface formation?

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The embryonic cuticle is a continuous hydrophobic layer essential during germination as it prevents water loss from the emerging seedling. The embryonic cuticle is formed *de novo* during seed development on the embryo surface, first as discontinuous patches, which subsequently merge to form a continuous layer surrounding the embryo. The GSO1/GSO2 signaling pathway controls this process via the detection of the endosperm-processed peptide TWS1. When signalling is perturbed, embryos produce discontinuous and clumpy cuticles, and the deposition of the endosperm-derived embryo sheath is lost, leading to seed twisting and poor seedling establishment. However, it remains unclear how proper embryo surface formation is achieved.

Reactive oxygen species (ROS) produced by NADPH oxidases participate downstream of signaling during other GSO1/GSO2 regulated processes, such as Casparian strip lignification. Preliminary results suggest that a production of ROS at the wild-type the embryo-endosperm interface may be lost in *gso1 gso2* mutants. Moreover, altering levels of ROS in the apoplastic space between the embryo and the endosperm, where the embryo cuticle and sheath are deposited, leads both to embryonic cuticle defects and impaired embryo sheath deposition, suggesting that, ROS are the likely effectors of the GSO1/GSO2 signaling pathway at the embryo surface.

Our efforts now are focused on unrevealing how ROS can orchestrate embryo surface formation. The embryo sheath, rich in extensins, may be key in this process. Extensin polymerization, which requires peroxidase activity using ROS as substrate, may act as a scaffold for the cuticle providing an organizing interface promoting uniform cuticle deposition.

P-23
Functional characterization of novel candidate peptide ligands for HAESA/HAESA-like receptor kinases

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Peptide ligands and their corresponding receptors are key players in modulating plant growth, development, and response to biotic and abiotic stresses. Members of the subfamily XI of leucine-rich repeat receptor kinases (LRR-RKs) recognise distinct secreted signalling peptides. While many of receptor-ligand pairs from this subfamily have been well studied, the HAESA-LIKE 3 (HSL3)-CTNIPs (also called NUT-SCREWs) receptor-ligand pair was only recently discovered (1, 2), whereas INFLORESCENCE DEFICIENT IN ABSCISSION/LIKE (IDA/IDL) peptides are ligands for phylogenetically-related HAESA (HAE)/HAESA-LIKE 1 (HSL1) /HAESA-LIKE 2 (HSL2)(3-5). In this study, we identified two previously undescribed candidate peptides that are phylogenetically interspaced between IDA/IDL and CTNIP ligand families and that are evolutionary conserved in many flowering plants. Preliminary data show that over-expression of the genes encoding these peptides show unique developmental phenotypes in *Arabidopsis thaliana*. In addition, transient expression of corresponding prepropeptides from *Nicotiana benthamiana* causes cell death in the native system. Results surrounding these phenotypes and on the genetic and biochemical characterisation of the perception mechanisms of these candidate peptides will be presented. Together, in addition to revealing potential novel peptide-receptor pairs and their functions, this work will provide insights into the evolution of ligand specificity within the subfamily XI of LRR-RKs.

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P-24
Functional analysis of target genes of MpCLE2 signaling in the stem cell zone of Marchantia polymorpha

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In the flowering plant *Arabidopsis thaliana*, overall morphology of the shoot apical meristem (SAM) is relatively constant throughout its lifetime. In contrast, the SAM of the liverwort *Marchantia polymorpha* undergoes periodic bifurcation in which the single apical cell is thought to be duplicated within the stem cell zone (SCZ). We have previously shown that MpCLE2 peptide, the single ortholog of *A. thaliana* CLV3/CLE40 peptides, positively regulates the size of the SCZ and the apical cell number, via the leucine-rich repeat receptor-like kinases, Mp- CLV1 and MpCIK (Hirakawa *et al.*, 2020; Takahashi *et al.*, 2021). To identify target genes of MpCLE2 signaling, we performed an RNA-seq experiment using gain-of-function alleles for MpCLE2 (MpCLE2-GOF) and loss-of-function of receptor genes (MpCLV1-LOF and MpCIKLOF). Among hundreds of differentially expressed genes, we found a NAC family transcription factor, namely MpJINGASA (MpJIN), whose expression level was dramatically decreased in MpCLE2-GOF. In qRT-PCR assays, MpJIN mRNA level was decreased in MpCLE2-GOF and was increased in MpCLE2-LOF, suggesting that MpCLE2 signaling inhibits the expression of MpJIN. Knockout alleles for MpJIN (MpJIN-KO) showed slight increase in the SCZ size, indicating that MpJIN negatively regulates the SCZ size under the MpCLE2 signaling. In promoter-reporter analysis, we found heterogenous MpJIN expression among cells in the SCZ: at the central position of the SCZ, the signal was almost at the background level while the signal was slightly higher in other cells in the SCZ. The signal was at the maxima in single cells flanking the SCZ, in which two cell layers are formed by a recent periclinal cell division. Consistently, we observed ectopic periclinal cell divisions in the SCZ in MpJIN-GR overexpression lines. Collectively, our data suggest that MpJIN acts as a negative regulator of stem cell identity by inducing periclinal division at the periphery of the SCZ. We are analyzing the details of MpJIN expression dynamics during dichotomous branching.

P-25
Large-scale production of modified plant peptide hormones

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Since the discovery of the first plant peptide hormone in the mid 90's, these peptides have made a considerable impact on our perception of inter plant cell signaling. More than 1000 putative peptides have been identified in Arabidopsis with multiple different functions. Many of the identified peptides are characterized by being heavily modified with e.g. tyrosine sulfation, glycosylations and proline hydroxylation. A specific group of peptides are essential for root growth and are defined by having one or two tyrosine sulfation in their relatively short sequences (between 5 and 18 residues). These sulfations are essential to the function of the peptides but are a major bottleneck in studies of these peptides, as they are very expensive to synthesize. To overcome this issue, we have utilized recent developments in non-canonical amino acid insertion using *E. coli*. By redirecting the amber stop-codon using a foreign tRNA loaded with sulfated tyrosine (sTyr), we ensure specific incorporation of sTyr in the correct position. We have focused on the PSY1 peptide, and are now able to produce bioactive sTyr containing PSY1 using standard *E. coli* growth conditions with yields on a scale that far exceeds what is reasonable from chemical synthesis. Our system makes it easy to produce similar peptides, as the E. coli strain developed is generally compatible with sTyr insertion, and thus the sequence of the expression construct could simply be swapped to a different peptide. Amongst other, this allows for hitherto unfeasible experiments requiring large peptide quantities to explore the peptides effects in an agricultural perspective.

P-26
A Novel Role for Phytosulfokine Signalling in Fumonisin B1 Toxicity

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In natural environments, plants are vulnerable to attack by disease-causing pathogens, including fungi, bacteria, viruses, nematodes, and protozoa. Plant pathogens pose a significant risk to agriculture, where they can reduce crop yields and quality, increasing production costs, and leading to economic losses for farmers. Fungi are a class of plant pathogens that cause more economic damage than any microorganisms. Fumonisin B1 (FB1) is a mycotoxin produced by the fungus *Fusarium verticillioides* that infects a range of crops. FB1 is the most prevalent fumonisin causing food contamination and, when it reaches the maximal concentration limit, is harmful to human health. Understanding the mechanisms underpinning plant stress-adaptive responses to FB1 is important in developing crops with robust disease resistance. Here, we identified a plant peptide hormone PHYTOSULFOKINE (PSK) and its receptor PHYTOSULFOKINE RECEPTOR 1 (PSKR1) as crucial *Arabidopsis thaliana* genes differentially expressed in response to FB1. We proposed that PSK signalling regulates Arabidopsis responses to FB1. We investigated the suspected role of PSK signalling in regulating FB1-induced cell death and elucidated the mechanism by which PSK signalling alters Arabidopsis responses to FB1.

P-27
Deciphering the signaling network in the overlap between pattern- and effector-triggered immunity

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Robust defense activation requires two distinct sets of immune receptors in Arabidopsis thaliana. Surface-exposed transmembrane proteins and intracellular receptors recognize extracellular patterns or intracellular microbe-delivered effectors, respectively. The interdependence of these signaling pathways has only recently been realized and remains to be fully understood (1,2). In particular, the composition and localization of protein complexes involved, as well as the hierarchy and nature of interaction between the affiliates need to be elucidated. And while nucleotide-based signaling compounds have very recently been implicated in effector-triggered immunity (3), their contribution to pattern-dependent signaling is not yet known.

To study these processes and characterize pattern-triggered post-translational modification of intracellular signaling components, we are adapting a recently developed split luciferase system (Nano-Glo/HiBiT peptide (4)), that allows detection of proteins of interest with both, exquisite sensitivity and specificity.

We will provide an update on the role of the small signaling molecules in pattern-triggered immunity, and present first results obtained with our new system for the detection of phosphorylation events in vitro and in vivo. Beyond, we will showcase other applications for the use of the HiBiT peptide tag, such as labeling and specific detection of proteins in plants and bacteria based on a set of new modules for the GoldenGate toolbox (5), and the development of a highly sensitive method for quantifying receptor/peptide-ligand interactions (6).

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P-28

Peptides with antimicrobial activity can be a good resource to tackle antimicrobial resistance in plants and animals. A case study with peptides identified from the Pisum sativum L. protein database

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Antimicrobial resistance has been on the rise in both plants and animals. A continuous use of antimicrobial agents (AMA) such as pesticides and antibiotics against pathogens creates selection pressure for the pathogens to mutate and evade the toxic effect. It prompts more usage of the AMA for their effectiveness, which results in a vicious cycle of higher doses, mutations, and microbial survival. The ever-increasing amount and number of pesticides released into the environment have put a question mark on the environmental and health safety of the practice. Similarly, in animals the reliance alone on antibiotics may not be sustainable. By using a bioinformatics approach, we developed a pipeline that can identify peptides from a large proteome database that potentially display properties of innate immunity. The latter is a basal form of defense that provides the first layer of protection. We analyzed pea proteomics data to generate possible fragments with 18 amino acids. For the pipeline, different algorithms or programs were combined. Duplicated fragments were removed by using the CD-Hit algorithm. The sequences were evaluated through five antimicrobial activity predictors: sense the moment, DBAASP, CAMP, ADAM and AxPep. The positively charged residue distribution, α-helix formation, and hydrophobicity of selected peptides were manually evaluated to further reduce the number. The top ten candidates from the curated list were evaluated for their toxicity against fungal, oomycete, and bacterial pathogens, which are the causative agents for many diseases in crops and animals. Depending on the pathogen, the peptides showed IC50 values in the range of 1-20 µM.

P-29
The role of cysteine oxidative modifications of Cysteine Rich Receptor Kinases in extracellular ROS perception.

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Plants produce Reactive Oxygen Species (ROS) in the apoplast as a universal stress response for intercellular signaling and stress adaptation. However, it is not known yet what are the apoplastic ROS sensors in plants and how ROS perception modulates protein-protein interactions and downstream signaling pathways. Here we show that the family of Cysteine Rich Receptor Kinases (CRKs) serves as a apoplastic ROS sensor. These receptors are characterized by two Domains of Unknown Function 26 (DUF26) in their extracellular domain (ECD) with a conserved cysteine motif (C-8x-C-2x-C). Our study shows that the cysteines in the ECDs of CRKs undergo oxidative modifications modulating protein-protein interactions and further cellular responses.

P-30
Endogenous FER-RALF signaling is involved in successful host colonization of powdery mildew on Arabidopsis thaliana

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With over 400 different species and nearly 10 000 possible host plants, powdery mildew is one of the most widespread fungal diseases. During the powdery mildew infection cycle, a constant interaction occurs between the fungus and the host plant which determines the infection success. Plant genes that support pathogen infection are termed susceptibility genes.

In Arabidopsis, the receptor kinase FERONIA (FER) was found to be a powdery mildew susceptibility gene as *fer* mutants are more resistant to infection. FER perceives endogenous RAPID ALKALINIZATION FACTOR (RALF) peptide ligands to control various aspects of plant growth, development and immunity. With a combination of peptide treatment, loss-of function and overexpression studies, we now show that RALF peptide maturation and perception by FER is required for successful powdery mildew infection on Arabidopsis. We hypothesize that powdery mildew fungi hijack endogenous RALF-FER signalling to adjust the apoplastic pH for creating a favourable environment for host colonization.

P-31
Decoding Plant Immunity: Unraveling Flg22-Induced Phosphorylation of the FLS2 Signaling Network

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Pattern recognition receptors (PRRs) play a key role in the first line of defence in plant immunity. PRR binding of their cognate ligands, so called pathogen-associated molecular patterns (PAMPs), triggers a signalling network that ultimately results in immunity. It has yet remained largely unanswered how activated PRRs are connected to downstream defence activation. However, it is evident that changes in phosphorylation and receptor-like cytosolic kinases (RLCKs), such as BIK1 and PBL1, play a major role. Here we report a temporal analysis of flg22-induced phosphorylation changes in Arabidopsis Col-0 seedlings, characterizing phosphorylation on over 3000 proteins. Using two complementary label-free quantification approaches, we quantified several thousand phospho-peptides and identified several hundred differential phosphorylation events on a wide range of proteins. This includes differential phosphorylation of proteins from the major families associated with PAMP-triggered immunity, e.g., receptor kinases, mitogen-activated protein kinases, calcium-dependent protein kinases and RLCKs. We also quantified previously characterised differential phosphorylation on several proteins, e.g., RBOHD, OSCA1.3, AHA1 and MAP4K6 (Benschop et al., 2007; Kadota et al., 2014; Thor et al., 2020), validating our approach. I will present several new potential signalling components upstream of MAP kinase cascade, will outline how this temporal phospho-proteome data set increases our understanding of the FLS2 signalling network and provides a major new resource for the plant community.

P-32
Phytocytokine signaling in maize immunity

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Phytocytokines are small peptides released via proteolytic processing from a pro-peptide and are thought to alarm bystander cells upon pathogen attack and other stresses. We have recently shown that phytocytokines and MAMPs trigger unique and antagonistic features of plant immunity, initially resulting in a partially similar activation of immune responses, but unlike microbial signals, upon cell damage maize phytocytokines do not induce cell death in the surrounding tissues. The maize-specific phytocytokine Zip1 triggers salicylic acid (SA) responses such as PR-gene expression and apoplastic papain-like cysteine protease (PLCPs) activation. Similar responses have been observed for the maize phytocytokines IRP and PSK1. In infection assays with two fungal pathogens of different lifestyles we found that these phytocytokines affect the development of disease symptoms, likely due to the activation of the SA pathway. Interestingly, during the course of cell death triggered by the avirulent bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo), secreting the effector molecule AvrRxo1, we have identified a small peptide that upon wounding can induce cell death in surrounding tissues. We aim to further characterize this novel peptide and its mechanism of activation and regulation of cell death. We now hypothesize that maize phytocytokines involved in the salicylic acid response are mostly triggering pro-survival signals to bystander cells as shown for Zip1 and IRP. To this end, we started a peptidomics screen to identify other small signalling peptides involved in the SA pathway. Overall, we aim to understand the mechanism leading to the divergence of signalling outputs between phytocytokines and MAMPs and the role of hormonal pathways in the decision-making "to live or to die".

P-33
LORE mediated immunity in Brassicaceae: mechanistic insights from natural diversity

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The receptor kinase LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION) is a pattern-recognition receptor that senses 3-hydroxy fatty acid metabolites, such as 3-hydroxydecanoic acid (3-HDA), released by Gram-negative bacteria (1,2). LORE alias SD1- 29 belongs to the class of S-domain-1 (SD1) receptor kinases. How S-domain receptor kinases are activated and trigger downstream signalling is largely unknown. To unravel the mechanism of 3-HDA sensing by LORE and activation of downstream immune signalling at the molecular level, we apply a combination of biochemistry, genetics, computational modelling, and natural diversity screening. I will present an overview of our current work on the natural diversity of LORE in Brassicaceae, LORE activation through dimerization and ligand binding.

(1) Ranf S, et al. (2015) A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in *Arabidopsis thaliana*. Nature Immunology 16:426-433.

(2) Kutschera A, et al. (2019) Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants. Science 364, 178-181.

P-34
Regulation of immune receptor kinase LORE by Calcium sensor Calmodulins (CaMs)

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The plant immune system is capable of actively recruiting various cell-surface immune receptors, when it perceives infection from pathogens (1). In pathogen-associated molecular pattern (PAMP) -triggered immunity (PTI), cell-surface receptor-like kinases (RLKs) serve as pattern recognition receptors (PRRs) which is responsible for recognizing pathogen elicitors. *Arabidopsis thaliana* S-domain-RLK LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION) is identified as PRR which sense 3-OH-C10:0 as ligand in antibacterial immunity (2, 3). Phosphorylation and the second messenger Ca2+ are universal signaling components in eukaryotes and also central to RLK signaling in plants. It is reported that LORE Y600 phosphorylation is important for stimulating the immune response towards 3-OH-C10:0 in *Arabidopsis* (4). Several RLKs from *A. thaliana* binds to calmodulin (CaM) or calmodulin-like protein (CML) family in a Ca2+-dependent manner and regulate RLK kinase activity (5, 6). It is hypothesized that LORE, along with CaM/CML forms a receptor complex and works in an orchestrated manner to facilitate the signaling mechanism in plants. The significance of this research would be ideal to discover the molecular machinery of immune receptor complexes in mediating pathogen perception and immunity activation in plants.

P-35
How Arabidopsis perceives insect eggs

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Insect eggs from the Large White butterfly *Pieris brassicae* release phosphatidylcholines (PCs) that trigger innate immunity in Arabidopsis, including salicylic acid accumulation, defense gene expression and localized cell death. These responses are mediated by candidate cell-surface Lectin Receptor Kinases (LecRK-I.1 and LecRK-I.8) and calcium channels. Whether PCs or derived metabolites bind to these LecRKs is currently under investigation. In addition, oviposition inhibits growth of pathogens through the establishment of a systemic acquired resistance, providing a potential bene t for hatching larvae.

P-36
Characterization of CrRLK1L-RALF modules during Arabidopsis powdery mildew colonization

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CATHARANTHUS ROSEUS RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) proteins play central roles during plant growth, development and immunity. Together with its endogenous RAPID ALKALINISATION FACTOR (RALF) peptide ligands, the CrRLK1L FERONIA (FER) positively regulates Pattern-Triggered Immunity (PTI) and antibacterial resistance by facilitating the formation of ligand-induced pattern recognition receptor (PRR) complexes. In contrast, during fungal powdery mildew infection FER functions as a susceptibility factor. While the underlying molecular mechanisms remain elusive, additional members of the CrRLK1L receptor family affect the Arabidopsis-powdery mildew interaction in opposite ways, suggesting functional diversification. On the other hand, the Arabidopsis genome encodes for at least 37 RALF peptide members with contrasting functions during PTI and growth. We will explore the functional diversity of Arabidopsis CrRLK1Ls and RALFs to elucidate context-specific signalling modules which may create transferable knowledge for other CrRLK1L-regulated pathways shaping plant development and immunity.

P-37
Developing tools to study the regulation of the immune receptor LORE

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Pattern Recognition Receptors (PRRs) are vital frontline components of the plant immune system which detect invading microbes through conserved microbe-associated molecular patterns (MAMPs) and activate Pattern-Triggered Immunity (PTI). The PRR LORE (LIPO-OLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION / SD1-29) recognises medium chain 3-hydroxy fatty acid (3-OH-FA) metabolites in *Brassicaceae* to initiate PTI (1,2). Previously, we investigated the natural variation in 3-OH-FA perception in *Brassicaceae* as well as the importance of LORE homomerization in the activation of downstream signalling (3). However, to date, few details are known about the precise regulation of LORE. We are therefore developing epitope-tagged versions of LORE to facilitate the identification of key post-translational modification sites and interaction partners. As C-terminally tagged versions show altered signalling kinetics, we are establishing a series of N-terminally tagged LORE transgenic lines, which from initial studies appear to show wild-type responses to 3-OH-FA. Transient expression of LORE in *Nicotiana benthamiana* results in cell death and low LORE protein levels likely due to spontaneous LORE homomerization and subsequent activation of PTI. To circumvent this obstacle, we are now developing the CLEAN-LORE (Controlled activation of LORE kinase signalling using receptor chimera) system. By fusing the LORE kinase domain to the transmembrane and extracellular domains of the EFR and BAK1 receptors, we hope to conditionally bring together and activate LORE kinase domains upon elf18 treatment.

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2. Kutschera, A. et al. Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants. Science (1979) 364, 178-181 (2019).

3. Eschrig, S. et al. LORE homomerization is required for 3-OH-C10:0 induced immune signaling. bioRxiv (2021) doi:10.1101/2021.09.27.461997.

P-38
Investigating the co-evolutionary history of the family-specific plant receptor kinase MIK2 and its SCOOP ligands

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Plant secreted signaling peptides regulate growth, development, and stress responses. Nevertheless, specific steps in the evolution of these peptides and their receptors are not well understood. Recent studies reported the characterization of the Brassicaceae-specific family of SERINE RICH ENDOGENOUS PEPTIDES (SCOOPs). In *Arabidopsis thaliana*, SCOOPs were shown to be transcriptionally induced during stresses and perceived by the leucine-rich repeat receptor kinase MALE DISCOVERER 1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2). *In silico* analysis of > 30 plant genomes within and outside the Brassicaceae family reveals the dynamic evolution of SCOOPs and a strong conservation of functional MIK2 homologues which are family-specific, suggesting the appearance of a common ancestral receptor ~31 mya. In addition, we leveraged AlphaFold-Multimer predictions across Arabidopsis MIK2/SCOOPs and the respective Brassicaceae homologues to identify candidate interaction sites for functional validation, in order to de ne evolutionary events leading to the sensing of sequence-divergent SCOOPs. Analysis of MIK2/SCOOP evolution and function provides a model for functional diversification of other plant receptors and their ligands.

P-39

A conceptual framework for the nanoscale regulation of receptor complexes

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Sensing of self, non-self, and modified-self molecules by cell surface receptors plays preponderant roles in regulating all aspects of plants life. Receptor kinases (RKs) are main ligand-binding cell surface receptors sensing and relaying signals at the cell surface. RKs forms ligand-induced complexes to initiate signaling events. For instance, upon perception of bacterial flagellin, the ligand-binding receptor FLS2 associates with its co-receptor BAK1 to initiate immune signaling. While the genetic and structural bases underlying ligand-induced formation of receptor complexes for archetypical signaling pathways is well documented, how their associations are regulated in space and time remain enigmatic. We used live cell imaging approaches, such as variable-angle total internal fluorescence microscopy, single-particle tracking, to analyse the organization and dynamics of FLS2 and BAK1 within the plasma membrane. We observed that the conditional association between FLS2 and BAK1 follows a defined and generalizable spatial and temporal logic and open ways toward the identification of the underlying regulatory mechanisms.

P-40
Holding everything together - HIR2, a key factor of receptor organization in plasma membranes?

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For the plant immune system, the initial recognition of pathogens represents a critical step that is mediated by intracellular and cell surface receptors. Transmembrane receptors at the plasma membrane are located in distinct nanodomains whose formation mechanism remains elusive. We identified HYPERSENSITIVE INDUCED REACTION 2 (HIR2) as a BAK1-INTERACTING RECEPTOR 3 (BIR3) interacting protein. HIR2 belongs to a SPFH domain-containing family that is supposed to function in membrane organization, it is enriched in nanodomains and upregulated upon pathogen treatment. We demonstrated its interaction with various ligand-binding receptors, including FLAGELLIN SENSING 2 (FLS2), BRASSINOSTEROID INSENSITIVE 1 (BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE (BAK1).

Responses to pathogen-associated molecular patterns (PAMPs) and brassinolide treatment are affected in *hir2* mutants implying that HIR2 contributes to proper receptor signaling. Using single-particle tracking photoactivated localization microscopy (sptPALM), we compared the dynamics of individual receptor proteins in Arabidopsis Col-0 and *hir2* mutant lines expressing receptors fused to the photo-switchable fluorophore mEos3.2. These lines serve as a powerful tool to investigate the impact of HIR2 on receptor dynamics and nanodomains organization within plasma membranes, thereby further refining our understanding of membrane nanodomain organization.

P-41
Identify the potential role of LORE paralogs in the 3-OH-FA-triggered immune response of Arabidopsis

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Plants have evolved a multi-layered immune system to sense and respond to microbial pathogens. The defense mechanism involves the ability to detect microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) and activate pattern-triggered immunity (PTI). In Brassicaceae, 3-hydroxylated fatty acid (3-OH-FA) metabolites are sensed by the S-domain (SD) receptor kinase LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION or SD1-29), which is localized on the plasma membrane. To date, homodimerization or heterodimerization with its homologous protein has shown to be essential for the immune response mechanism of SD-receptor-like kinases (RLKs). LORE belongs to a Brassicaceae-specific clade of SD-RLKs comprising four tandem-located SD-RLKs in Arabidopsis (LORE, SD1-23, SD1-27, SD1-30). These homologous genes share 67-79% of protein sequence similarity, in which SD1-23 is the closest paralog with LORE. However, SD1-23 seems not to be involved in sensing 3-OH-FA due to its wild-type-like immune responsiveness upon 3-OH-FA treatment. Transcriptional analysis revealed that SD1-27 and SD1-30 are co-regulated with LORE upon biotic stress treatments. To investigate the protein-protein interaction among LORE and its paralogs, LORE, SD1-27, and SD1-30 were co-expressed in combinations in *N. benthamiana* for FRET-FLIM interaction analysis. SD1-27 and SD1-30 could interact with LORE, suggesting a potential role in the LORE immune response machinery.

P-42
Using directed evolution approach to engineer recognition specificity of plant pattern recognition receptors

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Plants employ pattern recognition receptors (PRRs) to detect conserved microbe-associated molecular patterns and trigger immune responses. While the engineering of novel PRR recognition specificities has immense potential in enhancing plant disease resistance, progress has been hindered by limited protein design knowledge and low screening throughput. Here we will present our efforts to leverage the power of artificial evolution to engineer PRRs by developing a yeast-based system to screen or select desired PRR variants from a mutagenesis library. As a proof-of-concept study, we aim to engineer the flagellin receptor FLS2 receptor to gain recognition towards divergent flagellin-derived epitopes from pathogens that can otherwise evade plant perception.

P-43
Polar RhoGTPase signalling at the plasma membrane of germinating pollen grains

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Cells need to coordinate multiple processes and signalling pathways to accomplish polar growth. To set their growth direction these cells need to sense directional cues in their environment. Additionally, these cells must maintain a precise balance of growth and cellular integrity. Both processes are regulated, among others, by peptide-sensing RECEPTOR-LIKE KINASES (RLKs). We use germinating pollen grains of *Arabidopsis thaliana* to investigate the coordination of polar signalling pathways. Pollen grains establish polar signalling and growth domain de novo in a previously unipolar cell and thus provide a great model to investigate the onset of polar signalling and growth. Our investigations focus on RhoGTPases OF PLANTS (ROPS) and their regulating proteins, as this signalling pathway is downstream of most known RLK signals and is required to translate the sensed information into cellular reactions. We show that distinct ROP activators, which are regulated by RLKs, are required for pollen germination and thus initiation of a polar growth domain. Furthermore, we investigated the phosphoproteome of dormant and germinating pollen and describe the phosphorylation pattern of multiple growth regulators during pollen germination. With this, we could show that phosphorylation of ROPGEFs can render them active or inactive, dependent on the phosphorylation site, which inhibits or promotes pollen germination. Our research contributes to our understanding of signalling pathways and changes in protein activity downstream of RLK signalling.

P-44
A role of RLK7 in the IDA signaling pathway?

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The abscission of floral organs and emergence of lateral roots in Arabidopsis are regulated by the peptide ligand INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), the receptor protein kinases HAESA (HAE), HAESA-LIKE 2 (HSL2), and members of the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family. In addition to the cell separation processes, components of the IDA signaling pathway are found to regulate the expression of defense genes in the abscission zone (1) and to be working during *Pseudomonas* and drought induced cauline leaf abscission (2,3). *IDA* expression is up-regulated in response to various biotic and abiotic stresses such as salinity stress, wounding, and the bacterial elicitor flagellin (flg22). We have previously shown that, similar to flg22, IDA induces a receptor-dependent rapid release of cytosolic Ca2+ and the production of reactive oxygen species (ROS) (4). Also, a dual application of IDA and flg22 leads to an enormous upregulation of defense related genes in Arabidopsis seedlings (4).

In this project, we are investigating receptor complexes involved in regulating the IDA-induced defense response. By the use of protein interaction techniques such as FRET and extracellular interaction assays, we identify a receptor known to be involved in innate immunity, RECEPTOR LIKE KINASE 7 (RLK7), as an interaction partner to HSL2. IDA treatment promotes heteromerization between HSL2 and RLK7. Interestingly, *IDA* is upregulated in response to the endogenous plant peptide PIP1, known to amplify immunity in planta through the RLK7 receptor. We aim to investigate how RLK7 functions in combination with HSL2 to regulate the known IDA induced defense responses such as an increase in cytosolic Ca2+, ROS production, and upregulation of defense related genes.

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P-45

Antagonistic RALF peptides control an intergeneric hybridization barrier on Brassicaceae stigmas

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Pollen-pistil interactions establish interspecific/intergeneric prezygotic hybridization barriers in plants. Rejection of undesired pollen at the stigma is crucial to avoid outcrossing but can be overcome with support of mentor pollen. The mechanisms underlying this hybridization barrier are largely unknown. Here in Arabidopsis, we demonstrate that receptor-like kinases FERONIA/CURVY1/ANJEA/HERK1 and cell wall proteins LRX3/4/5 interact on papilla cell surfaces with autocrine stigmatic RALF1/22/23/33 peptide ligands (sRALFs) to establish a lock that blocks the penetration of undesired pollen tubes. Compatible pollen-derived RALF10/11/12/13/25/26/30 peptides (pRALFs) act as a key, outcompeting sRALFs and enabling pollen tube penetration. By treating Arabidopsis stigmas with synthetic pRALFs, we unlock the barrier, facilitating pollen tube penetration from distantly-related Brassicaceae species and resulting in interspecific/intergeneric hybrid embryo formation. Therefore, we uncover a 'lock-and-key' system governing the hybridization breadth of interspecific/intergeneric crosses in Brassicaceae. Manipulating this system holds promise for facilitating broad hybridization in crops.

P-46
Phosphorylation profiling of phytocytokine versus MAMP signaling reveals systemin-specific signaling events

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Plasma membrane-localized pattern recognition receptors (PRRs) percieve host-derived phytocytokines (e.g. systemin) and microbe-associated molecular patterns (MAMPs; e.g. flg22 and chitin). Upon ligand binding, activation of PRRs induces similar early phosphorylation-regulated responses including extracellular alkalization and reactive oxygen species burst. However, despite similar early signaling events, the downstream responses are distinct: MAMPs trigger plant innate immunity, and the wound response is elicited by systemin. Therefore, the question arises as to how systemin-mediated responses differ from that of MAMPs, and how specificity is achieved. To address this question, we applied phosphoproteomics in tomato wild type (WT) and systemin receptor-deficient (*syr1*) cell cultures to compare the early cellular responses to systemin, flg22, and chitin within a 45-minute time frame.

Overall 5262 phosphosites mapping 2299 proteins were detected by LC-MS/MS. Seven temporal phosphorylation patterns of phosphosites were grouped by K-means clustering. Interestingly, early transient dephosphorylation was identified as a hallmark of the systemin response in WT cells and was lost in *syr1*. A group of plant PP2C phosphatases was identified as a central hub in the network of systemin-specific phosphosites. The impact of phosphorylation on phosphatase activity was tested by phosphomimetic and phospho-null mutants *in vitro*. A specific role for PP2C phosphatases in early systemin responses (e.g. ROS burst and MAPK activation) was confirmed using CRISPR/Cas9 tomato mutants. Wounding and insect feeding assays are being used to validate the function of PP2C phosphatases in the systemin-induced wound response.

P-47
The regulatory mechanisms of the balance between pollen tube growth and ovular guidance

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In angiosperms, pollen tubes (PTs) will grow through a long journey from the surface of the stigma to the deeply embedded ovule in which the female gametophyte is enclosed to complete double fertilization. During this process, the growth direction of PT is finely guided by various signals. It is widely believed that pollen tube growth and guidance are closely associated, but how this association is regulated is not yet clear. Here in our preliminary study, we conducted phenotypic analysis of the mutant of a cysteine-rich peptide signal FGS that is specifically expressed at the transmitting tract in *Arabidopsis thaliana*. We found that WT pollen tube growth was compromised in *fgs* mutant pistils, but the sensitivity and the speed of the pollen tube guidance to the ovules were significantly increased. It seems that the potential receptors of FGS are possibly competing on perceiving FGS with pollen tube guidance signals, suggesting an "antagonistic" relationship between pollen tube growth and guidance in Arabidopsis.

P-48
Comparisons of two receptor pathways in a single cell-type reveal features of signalling specificity

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To respond appropriately to diverse stimuli, cells harbour numerous receptor pathways yet share downstream signalling components. Mitogen-Activated Protein Kinase (MPK) cascades, present in all eukaryotes, act as central hubs shared amongst diverse signalling pathways. How signalling specificity is maintained within a single plant cell-type is not well understood. We engineered a genetic background for direct comparisons of a developmental and an immunity pathway in the Arabidopsis root endodermis. We show the two pathways maintain distinct outputs despite similar MPK phosphorylation patterns. Systematic activation of all individual MPK Kinases (MKKs) in the endodermis revealed that different MKK groups can drive distinct outputs. We propose that specificity is achieved through a balance of combinatorial activation and cross-pathway inhibition within the MPK cascade. Our findings demonstrate the ability of MPK cascades to generate diverse outputs in one plant cell-type, providing new insights into the mechanisms contributing to signalling specificity.

P-49
AtTYT RLCKs regulate pollen tube growth by modulating apical calcium ion concentration

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Pollen tube growth is important for successful double fertilization in flowering plants. According to our RNA-seq data, we find a group of Arabidopsis receptor-like cytoplasmic kinases highly expressed in pollen and pollen tubes. GUS staining further verified their expression patterns and GFP-fused protein was localized on plasma membrane in apex and sub apex of pollen tubes. Quintuple knock-out mutant (*qui*) was generated and the competitiveness of qui pollen tubes severely declined compared to that of wild type (WT). Further study showed that qui pollen tubes grew more slowly in transmitting tract than WT but finally they could accomplish fertilization, that's why we name these genes *Take Your Time (TYTs)*. Further observations demonstrate that in *qui* pollen tubes, (Ca2+)cyt is largely reduced while tip-focused Ca2+ pattern remains. Besides, we find that increasing (Ca2+) in pollen germination medium can promote in vitro growth of *qui* pollen tubes, and *qui* pollen tubes are less sensitive to exogenous calcium application compared with WT, suggesting defects in calcium absorption in *qui*. Meanwhile, distribution pattern of actin filaments altered in *qui* pollen tubes. These results indicate that AtTYTs may modulate apical calcium concentration, and in turn affect arrangement of cytoskeletons to regulate pollen tube growth.

P-50
EC1, small cysteine-rich proteins secreted by the egg cell for sperm-egg interactions

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Double fertilization is a distinctive reproductive mechanism found in flowering plants, where male and female gametes are formed within haploid gametophytic generations (pollen and embryo sac). When a pollen grain has landed on a compatible stigma, it rehydrates and forms a pollen tube that grows rapidly towards the ovule, whose integuments enclose the embryo sac with the two female gametes (egg cell, central cell) and accessory cells (synergid cells, antipodal cells). After successful interaction with one of the synergid cells, the tip of the pollen tube bursts open and ejects a pair of sperm cells. The sperm pair thus enters the cleft between the egg and the central cell, where, in *Arabidopsis thaliana*, within about 5-10 min one sperm cell attaches to each of the two female gametes, the sperm pair separates, and one sperm cell fuses with the respective female gamete (1,2). When the sperm cells arrive at the fusion site, the A. thaliana egg cell secretes small cysteine-rich EC1 proteins from intracellular vesicle-like structures. This regulated secretion of EC1 induces the co-transport of the gamete fusogen HAP2 and the fusion facilitator DMP9 to the plasma membrane of sperm cells, enabling gamete fusion and, in particular, sperm-egg fusion (3,4). Loss of the five *EC1* genes in *A. thaliana* significantly impairs sperm adhesion and gamete fusion, resulting in a substantial reduction in seed formation (3,5). Unlike many other secreted cysteine-rich mini-proteins, EC1, which is characterized by a specific pattern of six cysteines and at least two characteristic sequence motifs, is highly conserved in flowering plants. Our progress in understanding the relationship between structure and function of EC1 proteins, its regulated secretion, binding to lipids and proteins, and the evolutionary innovation of the cellular processes induced by EC1 during seed plant reproduction will be presented.

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P-51
Function and evolution of EC1 and other ECA1 gametogenesis-related cysteine-rich miniproteins

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Secreted small cysteine-rich proteins (CRPs) are found in all multicellular eukaryotes, where they exert various functions such as antimicrobial peptides, as extracellular enzyme inhibitors, as modulators of ion channels, or as ligands for plasma membrane-localized receptor kinases. Angiosperms possess a striking abundance of functionally diverse CRPs. The family of Early Culture Abundant 1 (ECA1) gametogenesis-related CRPs has been reported to occur exclusively in angiosperms, including Arabidopsis thaliana and the basal angiosperm Amborella trichopoda. In A. thaliana their expression appears to be restricted to the embryo sac. Despite their surprising abundance, only the function of a small subfamily of ECA1 gametogenesis-related CRPs has been described. The EGG CELL 1 (EC1) subfamily comprises five proteins that have been shown to be functionally redundant and essential for sperm activation and fusion during double fertilization. When sperm cells are released from the pollen tube, EC1 proteins are secreted from the egg cell and trigger the relocation of the fusogen HAP2 and the fusion facilitator DMP9 from the sperm cell endomembrane system to the sperm cell plasma membrane, allowing gamete fusion. A Cas9-free CRISPR quintuple knockout of the five Arabidopsis EC1 genes results in major defects in gamete fusion. Sperm cells are unable to attach and fuse with the female gametes in the 5xec1 mutants, resulting in a severely reduced seed set. The functions of all other ECA1 gametogenesis-related CRP family members are still completely unknown. We aim to investigate the relationship between structure and function of EC1 and the role of other ECA1 gametogenesis-related CRP family members in the embryo sac of A. thaliana. Here we will present our studies on understanding the evolution of EC1 and EC1-related proteins in plants. To identify functionally relevant protein regions and understand the evolutionary innovation of EC1 and EC1-related proteins in seed plant reproduction, we are testing, among other things, the ability of mutant EC1 proteins and putative orthologs from angiosperms and gymnosperms to restore the reproductive phenotype in the Arabidopsis ec1 quintuple mutant.

P-52
Phytocytokine ignition: a ROS burst strategy against weeds

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In the context of sustainable agriculture and phytosanitary product restrictions, this study aims to take advantage of the diversity and specificity of plant small signaling peptides to manage weed plants by interfering with their Reactive Oxygen Species (ROS) regulation networks. The Black Nightshade species (*Solanum nigrum*) is a major but understudied adventive species, for which palliative control practices are the only alternatives to herbicide use. Therefore, we conducted an investigation to identify S. nigrum specific candidate secreted peptides, that could impede its development through a transcriptomic screening and pro ling approach. We performed a *de novo* reference transcriptome assembly using Trinity and EvidentialGene, combining long and short read sequencing technologies onto samples from multiple organs, developmental stages, and oxidative stress-inducing conditions (H2O2, Methylviologen, Atrazine, *Clavibacter michiganensis* and *Xanthomonas euvesicatoria* infections). The proteins deduced from transcript contigs were annotated using InterProScan and BLASTP against the proteomes of *S. lycopersicum* and *A. thaliana*. Transcripts encoding potential small secreted peptide precursors (shorter than 200 amino acids) were identified using SignalP and DeepLoc and/or by similarity to known secreted peptides. Subsequently, a differential RNA-seq analysis was conducted with previously described samples, resulting in a curated subset of 100 candidate peptide precursors, overexpressed in at least one of the applied stress conditions. This subset comprises previously unreported putative Solanaceae-specific secreted proteins, and a group of candidates has been selected for experimental validations. For each of them, the conserved motif predicted from sequence comparisons with homologs has been synthesized to evaluate their exogenous effects on *S. nigrum* seedling growth. This step is currently in progress and further studies are still required to demonstrate the potential of signaling peptides as selective bioherbicides for inhibiting weed growth in the field.

P-53
Monitoring the dynamics of the synergid-expressed receptor-like kinases during double fertilization

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Plants constantly sense and respond to their environment for proper vegetative and reproductive growth. Predominantly, they interpret their internal and external environment with the help of membrane proteins called receptor-like kinases (RLKs). FERONIA (FER) is an extensively studied plasma membrane-localized receptor kinase of the *Catharanthus roseus* RLK1-like (*Cr*RLK1L) family. It plays a multitude of physiological and developmental roles during plant growth and development, acting as a central hub coordinating complex intracellular and extracellular signals. *FER* gene was first identified to play a role as a female-specific regulator of fertilization. Two other synergid-expressed CrRLK1Ls: HERCULES RECEPTOR KINASE1 (HERK1) and ANJEA (ANJ), interact with FER and are also involved in PT-reception and double fertilization. HERK1 and ANJ form heterodimers and all three synergid-expressed *Cr*RLK1Ls localize to the membranes of the filiform apparatus facing the micropyle. Additionally, both *herk1* and *anj* mutants display a PT-overgrowth phenotype similar to *fer* mutants, revealing a probable redundancy in function. However, the specific role and sequence of their activation is still unknown. The study investigates the dynamics of these receptors by following their *in vivo* interactions using high-resolution live cell imaging during PT-reception and double fertilization. Therefore, the work aims to elucidate *Cr*RLK1L signaling in plants during the double fertilization process. Since FER is involved in such diverse processes as plant growth, innate immunity, pathogen resistance, hormone signaling, and reproduction, the project will set the stage for future studies and, in the long term, bring us one step closer to improved crops, and food security.

Keywords: Reproduction/ CrRLK1L receptor kinases / Fertilization / Pollen tube reception / High-resolution imaging

P-54
Tomato plants require an anti-systemin signal to balance four agonistic systemins for normal growth

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Systemin, the first peptide hormone to be identified in plants, functions in wound response and insect defense in tomato. We detected a factor in cultivated and wild tomato that specifically antagonizes the activity of systemin. Purification of this anti-systemin activity identified it as a systemin-like peptide constituting the C-terminus of a small protein. Tomato encodes three additional proteins with similar C-terms. However, these systemin-like C-termini are agonists like authentic systemin. Two different tomato mutants lacking anti-systemin exhibited pleiotropic phenotypes that progressively affected developmental processes from seed germination to fruit setting, suggesting a crucial role of anti-systemin to balance inadvertent wound responses induced via the four agonistic systemins during development.

P-55

The inoculation with Ensifer meliloti sv. rigiduloides improves considerably the growth of Robinia pseudoacacia under lead-stress

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Background and Aims

The objective of the present work was to characterize and identify the microsymbionts isolated from root nodules of *Robinia pseudoacacia* grown in the Zaida abandoned mine tailings.

Methods

After the isolation of bacteria from the plant root nodules, a REP-PCR was performed to eliminate duplicates. Subsequently, 16S rDNA sequence phylogeny and MLSA analysis using five housekeeping genes, as well as analysis of three symbiotic genes, were performed on the representative strains. Phenotypic analysis of the strains involved various physiological and biochemical tests.

Results

Twenty-seven isolates were obtained from the nodules, and REP-PCR grouped them into four clusters, from which 4 representative strains were selected. The 16S rDNA analysis and phylogeny as well as MLSA showed that 3 of the strains are affiliated with *Ensifer meliloti*, and the fourth strain is related to *Ensifer kummerowiae*. The symbiotic genes analyses proved that *R. pseudoacacia* is nodulated by two different symbiovars of *Ensifer* in these mine tailings.

Conclusion

To our knowledge, this is the first report on the isolation and phylogenetic identification of microsymbionts of *R. pseudoacacia* from lead-rich mine wastes in Africa.

Keywords: *Robinia pseudoacacia, Ensifer meliloti,* Bioremediation, Symbiovar rigiduloides

P-56
Characterization of a fungal tripeptide stimulating plant-fungus symbiosis

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Most land plants, including crops, are associated with arbuscular mycorrhizal fungi (AMF). During this symbiotic interaction, the plant acquires water and minerals from the fungal mycelium that explores the soil far beyond the plant root system. In return, the fungus gets sugars and lipids from the host plant. These exchanges occur in specialized inner root cortex cells of the plant, where the plant plasma membrane invaginates to create a plant-fungus interface, the arbuscule, made by dichotomus branching of fungal hyphaes.

To allow fungal colonization and proper establishment of the symbiosis interface, cell-cell communication is expected to play crucial roles. Indeed, we recently identified a CLE peptide gene in arbuscular mycorrhizal fungi that is strongly expressed during the symbiotic interaction (1), suggesting that the fungus itself might interfere with plant CLE-dependent processes within root tissues. This observation prompted us to search for additional gene encoding small secreted peptides in AMF genomes (2). In the model species *Rhizophagus irregularis*, we identified a family of five proteins predicted to deliver multiple copies of a tripeptide. Functional study of this gene family is ongoing. Our first results indicate that these fungal peptide genes are induced by plant exudates, are strongly expressed during symbiosis and can be detected by targeted mass spectrometry. Furthermore, exogenous application of the synthetic peptide increases mycorrhizal interaction in the model plant species *Medicago truncatula*, suggesting that these tripeptides might be new important players in symbiosis establishment and/or control.

(1): Le Marquer et al., 2019 New Phytologist

(2) : Le Marquer et al., 2019 BMC Genomics

P-57
Signaling peptides regulating nitrogen-fixing symbiotic nodulation in legumes

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Legume plants growing in mineral nitrogen (N) deficit develop a specific root organ in response to rhizobium symbiotic bacteria, the nitrogen-fixing nodule. Long distance (systemic) root-to-shoot-to-root regulatory pathways coordinate the number of nodules formed through signalling peptides perceived by Leucine-Rich Repeats Receptor-Like Kinases. In the *Medicago truncatula* model legume, C-TERMINALLY ENCODED PEPTIDEs (CEPs) are critical to allow root competence for nodulation when mineral N availability is limited, through the COMPACT ROOT ARCHITECTURE 2 (CRA2) receptor acting in shoots[1-2]. To optimize the number of nitrogen-fixing nodules depending on plant needs, another independent systemic pathway involving CLAVATA-LIKE (CLE) peptides and the SUPER NUMERIC NODULE (SUNN) receptor is additionally involved in shoots[3-4]. Furthermore, high levels of mineral N also activate this systemic CLE/SUNN pathway[5]. We showed that these positive and negative systemic pathways are dynamically coordinated 1) in roots by the action of cytokinin hormones acting through the CYTOKININ RESPONSE 1 (CRE1) receptor and the NODULE INCEPTION (NIN transcription factor), leading to the regulation of the production of speciffc CLE and CEP signalling peptides[6-7]; and 2) in shoots downstream of the CRA2 and SUNN receptor by regulating antagonistically the production of the miR2111 shoot-to-root systemic effector promoting nodulation[5,8]. The NIN-LIKE PROTEIN 1 (NLP1) transcription factor also contributes to the coordinated regulation of specific CLE and CEP peptides depending on N availability[5,9]. Currently, we are interested in 1) integrating other environmental factors modulating the CEP/CRA2 systemic pathway, 2) identifying shoot and root downstream effectors and 3) expanding studies to other signaling peptides involved in local regulations of symbiotic root and nodule development. Progress on the analysis of some of these symbiotic regulations will be reported.

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P-58
Sequence adaptation of Symbiosis Receptor-like Kinase (SymRK) enabling nitrogen-fixing root nodule development

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Root endosymbiosis is established between plant roots and microorganisms and involves the intracellular accommodation of fungi or bacteria to enhance acquisition of essential nutrients from the soil. Arbuscular Mycorrhiza (AM), formed with *Glomeromycota* fungi, is present in almost 80% of land plants and dates back to 450 million years ago. Root Nodule Symbiosis (RNS) emerged more recently, around 60 million years ago, and it is restricted to four plant orders, Fabales, including the model species *Lotus japonicus* (*Lj*), Fagales, Cucurbitales and Rosales (1). Despite the different evolutionary origin, AM and RNS require a set of conserved genes indispensable for symbiosis, the so-called Common Symbiosis Genes (CSGs) (1). It has been hypothesized that RNS evolved by co-opting CSGs already present in the more ancient AM. The only membrane-bound product of CSGs is Symbiosis Receptor-like Kinase (SymRK) from the family of Malectin-like Domain Leucine-Rich Repeat RKs (2,3). Transgenic complementation of *Lotus japonicus symrk* mutant with SymRK orthologs from species that form AM, but not RNS *e.g.* rice and tomato, driven by the endogenous *LjSymRK* promoter could restore AM but not RNS. This led us to hypothesise that during evolution SymRK underwent super-functionalisation: new features evolved that are necessary for the establishment of RNS, while maintaining the conserved function for AM (4,5). We performed a series of functional complementation assays with chimeric SymRK variants between tomato and *Lotus* to determine that the super-functionalization process is encoded in the intracellular part of the protein. In this project, we aim to explore the protein sequence diversity among SymRK orthologs, to pinpoint critical amino acid changes in *Lotus japonicus* SymRK intracellular domain, necessary for the establishment of RNS. We will study the mechanistic consequences of these adaptations at a cellular and molecular level by combining Cas12-genome editing tools, diverse -omics and biochemical approaches.

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P-59
Stress-induced calcium signaling in Arabidopsis thaliana-Bacillus rhizobacteria interactions

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Stress-induced calcium signaling in *Arabidopsis* has garnered significant attention over the past due to its pivotal role in facilitating adaptive mechanisms[1]. Recently, calcium signaling has been also found to take part in the process of biofilm formation in several *Bacillus* species, a necessary process for successful root colonization and establishment of plant-microbe interactions[2]. *Bacillus* rhizobacteria have been shown to have a role in growth promotion and stress resilience of plants raising further the interest in investigating the intricate plant-rhizobacteria interactions and especially its relevance as a critical factor in the plant response to environmental stress[3].

Here, we used new fast kinetics genetically encoded calcium biosensors to visualize calcium signaling, dynamics and fluxes with high spatial and temporal resolutions[4]. Biosensors were employed across systems for investigating stress-induced calcium signaling interplays that are occurring in *Arabidopsis thaliana-Bacillus* rhizobacteria interactions. Using quantitative biosensor imaging in combination with microfluidic devices, we were able to study abiotic stress response of a plant-microbe system[5].

Our results suggest key roles of calcium signaling in abiotic-stress responses for both *Arabidopsis thaliana* and its *Bacillus* rhizobacteria.

On the plant side, we have observed long-distance shoot-to-root calcium signatures which might be mediated by the plant-microbe interactions. How plant-microbe interactions may improve plant resilience to abiotic stress?

On the microbe side, we have observed a potential role of calcium signaling in response to abiotic stress. What mediates calcium signaling in Bacillus rhizobacteria? How calcium signaling may facilitate interactions of *Bacillus* rhizobacteria with *Arabidopsis* roots?

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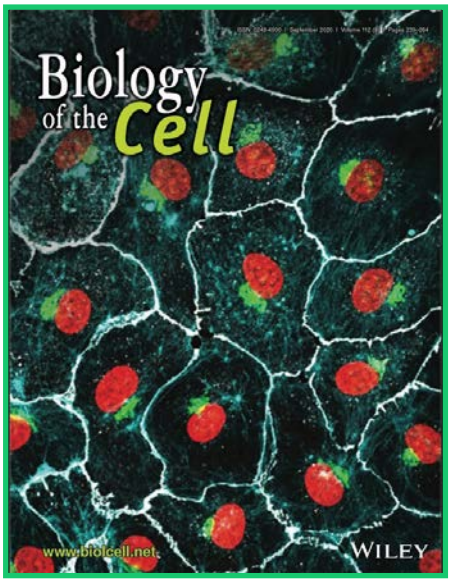

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