PLANT PEPTIDES AND RECEPTORS MEETING 2024

Conference booklet

WAGENINGEN
UNIVERSITY & RESEARCH

Welcome to the Plant Peptides and Receptors Meeting 2024

The **12th Plant Peptides and Receptors Meeting** takes place from the **11th to the 13th of September 2024** and is held at the Fletcher Hotel De Wageningsche Berg in **Wageningen**.

The conference targets interested Master's and PhD students, postdoctoral researchers, principal investigators, and non-academic participants. The workshop focuses on plant peptides, their membrane-bound and cytoplasmic receptors, their modifying enzymes, and downstream signalling events in plant development and adaptation. It will feature a number of invited speakers (link), talks chosen from abstract submissions, andPoster sessions. For more information, see the program.

The PPRM's primary goals:

- Highlight recent advancements in the field of plant receptor kinases
- Present different views and models of receptor activation, other than only canonical ligand perception
- Share technological advancements on how to conduct comprehensive mechanistic studies of RKs signalling
- Create a safe and inclusive environment for a scientific exchange
- Provide a platform for information exchange and potential future collaboration
- Take advantage of existing knowledge and expertise to develop interdisciplinary collaborative project ideas

The Organizing Committee

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Matthieu Joosten, WUR

Jasper Lamers, WUR

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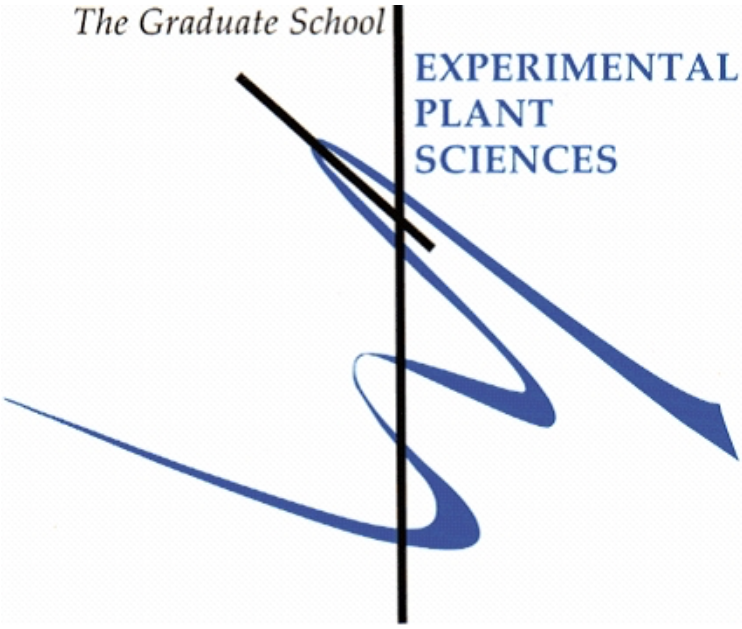
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Program

Wednesday, September 11th

11:30 – 12:00 Registration

12:15 – 13:00 Lunch

13:00 – 13:15 Opening of the meeting – Welcome by Elwira Smakowska-Luzan

Session I: Immunity

Chair: Kyle Bender

13:15 – 13:45 ***Keynote Speaker: Libo Shan***
Nature's Sentinel: Functional Insights and Evolutionary Patterns in Plant Peptide-Receptor Immune Signaling
13:45 – 14:00 ***Hannah Weber***
If you like it, put a ring on it – How HIR2 could organize RLKs in the plasma membrane
14:00 – 14:15 ***Cyril Zipfel***
A peptide-receptor module links cell wall integrity sensing to pattern-triggered immunity
14:15 – 14:30 ***Henriette Leicher***
Endogenous RALF peptide function is required for powdery mildew host colonization
14:30 – 14:45 ***Frank Menke***
Connecting Membrane Receptor Signalling And Effector-Triggered Immunity Via Helper NLR Phosphorylation
14:45 – 15:00 ***Oliver Johanndrees***
Evolutionary insights into the CTNIP-HSL3 Signaling Module Across Angiosperms
15:00 – 15:15 ***Ralph Hückelhoven***
Overlapping and distinct recognition specificity for exogenous and endogenous signalling peptides in MIK2-family receptors from different plant lineages

15:15 – 15:30 Flash talks: Even numbers - part 1 (6 talks)

15:30 – 15:55 Coffee Break

15:55 – 16:00 Pitch presentation by Thermo Fisher Scientific

Session II: Reproduction

Chair: Lei Wang

16:00 – 16:30 ***Keynote speaker: Li-Jia Qu***
Peptide/receptor-mediated signaling controls double fertilization in Arabidopsis
16:30 – 16:45 ***Zachary Nimchuk***
Floral innovation in the Asteraceae evolved through modifications in plant stem cell peptide signaling

16:45 – 17:00 ***Philipp Denninger***
Beyond receptors – Regulation of RhoGTPase signaling during pollen germination
17:00 – 17:15 ***Isaia Vardanega***
On the role of stem cell regulators in shaping barley spike architecture
17:15 – 17:30 ***José Antonio Montano García***
RALF/LRX implication in cell wall integrity during tomato fruit formation
17:30 – 17:45 ***Christian Hardtke***
Antagonistic CLE peptide pathways shape root meristem tissue patterning

17:45 – 18:00 Flash talks: Even numbers - part 2 (6 talks)

18:00 – 18:15 Pitch presentation by Molecular Plant

18:45 – 20:00 Dinner

20:00 – 22:00 Poster session I (even numbers) with drinks

Thursday, September 12th

Session III: Stress signalling and mechanosensing

Chair: Jasper Lamers

08:30 – 09:00 ***Keynote Speaker: Nora Gigli Bisceglia***
Exploring the Role of Cell Walls in Controlling Stress Responses in Plants
09:00 – 09:15 ***Irene Guzmán-Benito***
PBLs as Novel Players in Single-Cell Plant Signaling Specificity
09:15 – 09:30 ***Luiselotte Rausch***
Phytosulfokines as rapid cell wall mediators during root growth in Arabidopsis thaliana
09:30 – 09:45 ***Feng Yu***
Regulated Cleavage and Translocation of FERONIA Control Immunity in Arabidopsis Roots
09:45 – 10:00 ***Kris Vissenberg***
Rapid Alkalinization Factor 22 (RALF22) has a structural and signalling role in Arabidopsis root hair cell wall assembly
10:00 – 10:15 ***Jasmin Kemppinen***
Exploring the role of GHR1 in plant immunity: insights from Arabidopsis mutants with constitutively open stomata
10:15 – 10:30 ***Rong Li***
Poltergeist-Like 2 (PLL2)-dependent activation of the wound response distinguishes systemin from other phytocytokine signaling pathways

10:30 – 10:45 Flash talks: Odd numbers - part 1 (6 talks)

10:45 – 11:15 Coffee Break

Session IV: Complex formation and technical advancement

Chair: Martin Stegmann

11:15 – 11:45 Keynote: Herman Höfte
Pectin-protein interactions in plant cell wall assembly and expansion
11:45 – 12:00 ***Sergio Martin-Ramirez***
Redox-dependent extracellular interaction networks of Cysteine-rich Receptor-Like Kinases
12:00 – 12:15 ***Chuanyou Li***
Peptide REF1 is a local wound signal promoting plant regeneration
12:15 – 12:30 ***Lin Xi***
QSK1 modulates ER-PM contact sites to regulate small molecules transport through PD
12:30 – 12:45 ***Ann-Kathrin Rößling***
Mechanosensing and Signalling: The interplay of FERONIA, pectin, and RALF peptides in plant cell walls
12:45 – 13:00 ***Matthieu Joosten***

Immune signalling by cell surface-localised receptor-like proteins (RLPs) reveals its secrets

13:00 – 13:15 Flash talks: Odd numbers - part 2 (6 talks)

13:15 – 14:00 Lunch

14:00 – 18:00 Trip to Kröller-Müller Museum

18:30 – 20:00 Conference Dinner

20:00 – 21:30 Poster session (Odd Numbers) with drinks

21:30 Party

Friday, September 13th

Session V: Symbiosis

Chair: Kate Parys

08:30 – 09:00 ***Keynote Speaker: Simona Radutoiu***
Recognition of symbionts and pathogens by LysM receptor kinases
09:00 – 09:15 ***Yusuke Saijo***
Immune peptide receptor and symbiosis regulator drive mutualistic interactions with plant growth promoting bacteria in rice
09:15 – 09:30 ***Rafael Jorge León Morcillo***
RAPID ALKALINIZATION FACTOR 22 regulates root hair growth in response to fungal ethylene emissions
09:30 – 09:45 ***Emma Guillierme***
Balancing nodule number with a CLV1-like receptor: identification of signalling proteins using a proteomics approach
09:45 – 10:00 ***Sona Pandey***
Receptor kinase dependent G-protein phosphorylation and its role in regulating signaling during soybean nodulation

10:00 – 10:30 Coffee Break

Session VI: Development I

Chair: Svenja Augustin

10:30 – 11:00 ***Keynote: Charlotte Kirchhelle***
Cell wall perception on edge - translating cell geometry into directional growth
11:00 – 11:15 ***Kaltra Xhelilaj***
A plasma membrane pH-stat coordinates cell surface processes and root development
11:15 – 11:30 ***Ora Hazak***
Progression in xylem maturation relies on a non-cell-autonomous peptide-dependent mechanism
11:30 – 11:45 ***Peter Grones***
Plasma membrane alterations during plant immune response

11:45 – 12:30 Lunch

Session VII: Development II

Chair: Ran Lu

12:30 – 13:00 ***Keynote: André Kuhn***
RAF-like kinases are conserved intracellular integrators of rapid responses
13:00 – 13:15 ***Max Fishman***
RGF peptides and their cognate receptors play a role in Phtheirospermum japonicum haustorium development

13:15 – 13:30 ***Noel Blanco-Touriñán***
The brassinosteroid receptor gene BRI1 safeguards cell-autonomous brassinosteroid signaling across tissues

13:30 – 14:00 Poster and talk prizes and closing remarks

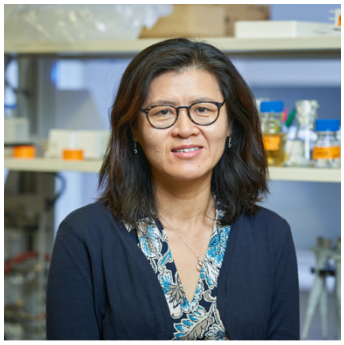
Invited speakers

Herman Höfte, INRAE, France
Title: Pectin-protein interactions in plant cell wall assembly and expansion.

Charlotte Kirchhelle, ENS de Lyon, France
Title: Cell wall perception on edge - translating cell geometry into directional growth.

André Kuhn, WUR, Netherlands
Title: RAF-like kinases are conserved intracellular integrators of rapid responses

Simona Radutoiu, Aarhus University, Denmark
Title: Recognition of symbionts and pathogens by LysM receptor kinases
The EMBO Keynote Lecture



Libo Shan, University of Michigan, US
Title: Nature's Sentinel: Functional Insights and Evolutionary Patterns in Plant Peptide-Receptor Immune Signaling

Li-Jia Qu, Peking University, China
Title: Peptide/receptor-mediated signaling controls double fertilization in Arabidopsis.

Nora Gigli Bisceglia, Utrecht University
Title: Exploring the Role of Cell Walls in Controlling Stress Responses in Plants

Social event (September 12th, Thursday afternoon)

DE HOGE VELUWE NATIONAL PARK and THE KRÖLLER-MÜLLER MUSEUM

Hoge Veluwe National Park is located in the central part of the Netherlands. The park covers 5,500 hectares of woodland, heath, grasslands and shifting sands and is the natural habitat for deer, mouflon and wild boar. On foot or on one of the free White Bicycles, you are free to roam around in nature. The free White Bicycles (1,800 in total) are stationed at the three entrances to the park, at the visitors' centre and at the museum.

The park also provides visitors with an array of cultural encounters. Jachthuis Sint Hubertus manor and the Kroller-Muller Museum are two of the more popular attractions within the park.

JACHTHUIS SINT HUBERTUS

Jachthuis Sint Hubertus is the former country house of Mr and Mrs Kröller-Müller. This 'total artwork' by architect H.P. Berlage is one of the Netherlands' most important monuments.

THE KRÖLLER-MÜLLER MUSEUM

At the Kröller-Müller Museum, you will find the world's finest Vincent van Gogh collection and enjoy masterpieces by modern masters such as Claude Monet, Georges Seurat, Pablo Picasso and Piet Mondriaan. The museum is a treasure trove of De Stijl and futurism, and will surprise you with presentations of contemporary artists. In the sculpture garden, you will stroll past works by Barbara Hepworth, Jean Dubuffet, Marta Pan, and others. For architecture enthusiasts, we have the museum buildings by Henry van de Velde and Wim Quist, the Gerrit Rietveld pavilion, and the Aldo van Eyck pavilion. Its location in the heart of De Hoge Veluwe Park and the unique combination of art and nature make the Kröller-Müller a place to enjoy and unwind.

ABSTRACTS

Invited talks

How does RALF22-mediated feedback signaling coordinate RALF22-mediated cell wall assembly in root hairs?

Afonso S, Schoenaers S, Faucher E, Wang Y, Lee H, Levasseur T, Bonnin E, Gonneau M, Vissenberg K, Peaucelle A, Cathala B, Santiago J, Haas K and **Höfte H**

A central unanswered question in plant biology is how plant cells expand while maintaining the integrity of their walls. Pectins, the main charged cell wall polysaccharides, play a critical role in this process. Pectin charge is regulated by pectin methylesterases (PMEs), which were shown to promote cell expansion in many developmental contexts. To investigate the underlying biophysical mechanism of this growth promoting effect, we are using Arabidopsis root hairs as a convenient mechanically-isolated model system. Root hairs show periodic variations in growth rate, cytosolic calcium levels, exocytosis and surface pH. This is expected to direct periodic pectin delivery and de-methylesterification, as reflected in the spatial periodicity of the cell wall organization. In this context, we show that the root hair-specific Rapid Alkalinisation Factor (RALF) 22 has a structural role since it forms a complex with Leucin Rich Repeat eXtensin (LRX) proteins and pectin, leading to the condensation of the pectin into a filamentous network. This network is essential for the integrity of the cell wall, but maintains the ability to expand. The function of RALF22 signaling through the LLG1-FERONIA complex can now be understood in the context of the coordination of the cell wall assembly process. We will show how detailed kinematic analysis provides new insights in this process.

References:
Schoenaers et al. 2024 Nature Plants, DOI : 10.1038/s41477-024-01637-8
Moussu et al. 2023 Science, DOI : 10.1126/science.adi4720

Cell wall perception on edge - translating cell geometry into directional growth

Charlotte Kirchhelle

Morphogenesis of multicellular organs requires coordination of cellular growth. In plants, cells are fixed in their position by their shared cell wall - consequently they have to integrate tissue-scale mechanical stresses arising through growth in a fixed tissue topology. To accomplish this, plant cells monitor cell wall mechanical status and adapt growth accordingly. However, while cell wall sensors have been identified, it is not clear how cell wall status is translated into directional growth. In this talk, I will present our latest work proposing that plants use their cell edges to translate cell wall feedback into directional growth. We describe two Receptor-Like Proteins, RLP4 and RLP4-L1, which occupy a unique polarity domain at cell edges established through a targeted secretory transport pathway. We show that RLP4s associate with the cell wall at edges via their extracellular domain and respond to changes in cell wall mechanics, and contribute to directional growth control in Arabidopsis.

Title: RAF-like kinases are conserved intracellular integrators of rapid responses

André Kuhn

Plants have evolved impressive capacities to fluctuating conditions in their surroundings. This is necessary since plants cannot relocate to escape unfavorable conditions but have to mount defensive and adaptive responses where they are rooted. These responses are only adequate if they are matched to the incoming signal. Prolonged inputs are met with slow responses, for example de-novo organogenesis; these invariably involve gene regulatory networks that take hours to days to manifest. In contrast, acute inputs must be met with fast responses, for example those that lead to rapid acclimatization of cellular physiology, which complete in mere seconds to minutes. Auxins function as a signaling molecules regulating both slow developmental and fast cellular response in plants. While developmental auxin responses are well-studied, it remained enigmatic how auxin can trigger fast cellular events. Moreover, it is entirely unknown how auxin signals are integrated in algae. We discovered that the auxin, when exogenously applied, triggers rapid (within 30s) proteome-wide phosphorylation in land plants and algae. We show that this phosphorylation response relies on the action of an evolutionary conserved group of RAF-like kinase (RAFs) that regulate physiological cellular processes e.g. cytoplasmic streaming. This suggests that RAFs play a critical and deeply conserved role in fast auxin responses. Further our data and those of others suggest that RAFs are a cornerstone of critical signaling modules in the cell and have been implicated in the integration of numerous environmental signals. This makes them an ideal platform to study the nature and origin of fast signal processing in plants.

Understanding the mechanism by which LysM receptors can control both immune and symbiosis signalling in plants

Magdalini Tsitsikli, Bine W. Simonsen, Maria M. Larsen, Camilla G. Andersen, Kira Gysel, Kasper R. Andersen, **Simona Radutoiu**

Receptor-like kinases (RLKs) are watchmen on duty at the membrane of plant and metazoan cells. They recognize a signal, interpret it, and initiate a corresponding intracellular response. In plants, RLKs control plant development and responses to symbiotic or pathogenic microbes. LysM RLKs are plant inventions that emerged early during their evolution and enable the perception of glycan ligands of microbial origin. Importantly, these receptors are able to distinguish between beneficial and pathogenic types of glycans and mount a symbiotic or immune response, accordingly. A key receptor present in all plants is CERK which enables the recognition of chitin (CO6-8) released by pathogenic fungi and the activation of immunity. Legume plants evolved Nod factor receptors (NFR) that recognize lipochitooligosaccharides (LCOs) with high specificity and sensitivity enabling symbiosis with nitrogen-fixing bacteria. We have identified how legume LysM receptors evolved specific LCO perception and how chitinous signals are distinguished at the molecular level is currently unknown. Next, we examined the functional role and biochemical properties of chimeric CERK6-NFR1 receptors and identified distinct signatures in the intracellular regions to be required and sufficient for determining immunity and symbiotic signalling in Lotus roots. Our findings provide a molecular framework for deciphering specific recognition of COs in plants and for engineering downstream signalling leading to root nodule symbiosis.

Nature's Sentinel: Functional Insights and Evolutionary Patterns in Plant Peptide-Receptor Immune Signaling

Libo Shan

Plant genomes encode over thousands of small peptides, some of which have been shown to be secreted with immunomodulatory functions, later referred to as phytocytokines due to shared features with cytokines in mammalian immunity. The phytocytokines can function as short- and long-distance defense signaling molecules and amply the immune responses triggered by microbial patterns and pathogen effectors via acting on the same target cell, adjacent cells, or distant cells. In addition, some phytocytokines could modulate plant physiological processes. Meanwhile, pathogens could mimic plant endogenous peptides to promote parasitism. I will present data to discuss the perception and mode-of-actions of phytocytokines perceived by cell surface receptors in plant-microbe interaction and environmental stress adaptations. I will also discuss the pathogen-host evolutionary mimicry of immunomodulatory phytocytokines.

Antagonistic RALF peptides control an intergeneric hybridization barrier on Brassicaceae stigmas

Zijun Lan*, Zihan Song, Zhijuan Wang, Ling Li, Yiqun Liu, Shuaihua Zhi, Ruihan Wang, Jizong Wang, Qiyun Li, Andrea Bleckmann, Li Zhang, Thomas Dresselhaus, Juan Dong, Hongya Gu, Sheng Zhong, **Li-Jia Qu**

Pollen-pistil interactions provide interspecific/intergeneric prezygotic hybridization barriers in plants. Rejection of undesired pollen at the stigma is critical to prevent outcrossing but can be overcome with support of mentor pollen. The mechanisms underlying this hybridization barrier are largely unknown. Here in Arabidopsis, we show that FERONIA/CURVY1/ANJEA/HERK1 receptor-like kinases and LRX3/4/5 cell wall proteins interact at the surface of papilla cells 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), as a key, outcompete sRALFs allowing pollen tubes to penetrate. Treatment of Arabidopsis stigmas with synthetic pRALFs unlocks the barrier and allows penetration of pollen tubes from distantly-related Brassicaceae species leading to the formation of interspecific/intergeneric hybrid embryos. Therefore, we uncover a 'lock-and-key' system that controls hybridization breadth of interspecific/intergeneric crosses in Brassicaceae. Manipulation of this system has the potential to facilitate wide hybridization in crops.

Exploring the Role of Cell Walls in Controlling Stress Responses in Plants

Nora Gigli-Bisceglia

During my talk, I will guide you through the role of plant cell walls in controlling stress responses in plants. I will summarise our research and link it to the current work being performed in my lab. I will present our recent findings on how salt alters the structure of cell walls and highlight the role of identified receptors crucial for maintaining cell wall integrity in controlling salinity stress. Additionally, I will share new, unpublished data suggesting the involvement of small signalling peptides that play opposite roles in controlling plant development as well as salinity stress. Overall, my talk will provide an overview of our current understanding of plant stress responses from a different perspective, proposing that one of the main drivers of stress responses may be the perception of cell wall stress.

Selected talks

Session I: Immunity

If you like it, put a ring on it – How HIR2 could organize RLKs in the plasma membrane.

Hannah Weber*, Alexandra Ehinger, Sven zur Oven-Krockhaus, Raffaele Manstretta, Julien Gronnier, Klaus Harter, Birgit Kemmerling

Receptor-like-kinases (RLKs) are essential for plant immunity. They are located at the plasma membrane (PM) in distinct nanodomains. To date, little is known about the factors involved in their organization. We identified HYPERSENSITIVE INDUCED REACTION 2 (HIR2) as an interaction partner of BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) interacting receptor 2 (BIR2), BIR3 and other RLKs. HIR2 belongs to the stomatin/prohibitin/flotillin/HflKC (SPFH) protein family and is enriched in nanodomains. While HIRs are found only in plants, the other members of the SPFH family are conserved in all domains of life. SPFH proteins likely act as a scaffold at various cellular membranes such as the mitochondrial, endoplasmic reticulum and plasma membrane. The molecular mechanism underlying this function is not yet fully understood, but recent studies were able to resolve the structure of two SPFH proteins, HflKC and flotillins, with the use of cryo-EM. They form large, ring-like oligomers that can entrap their cargo (protein) and may thereby organize membrane-domains.
Our Alphafold predictions showed that HIR2 can also form a ring-like structure with a cap consisting of two helices at the C-terminus. These rings might provide a platform for RLK-related signalosomes. Indeed, using single-Particle Tracking Photoactivated Localization Microscopy (sptPALM) to observe RLKs fused to the photo-switchable fluorophore mEos3.2, we found that the loss of HIR2 alters their dynamics, supporting its role as a scaffold in RLK signaling.

A peptide-receptor module links cell wall integrity sensing to pattern-triggered immunity

Keran Zhai, Jack Rhodes, **Cyril Zipfel***

Plant cell walls are highly dynamic structures, playing a critical role in diverse biological processes, including cell morphogenesis and plant-pathogen interactions. During these processes, plant cell wall integrity (CWI) can be impaired. Thus, it is important for plants to monitor consistently the state of their cell walls and ensure their functional integrity. Several components, such as receptor kinases, receptor-like proteins or mechanosensitive channels have been proposed in the past decade to act as CWI sensors in Arabidopsis. Here, we identify a specific ligand-receptor module responsive to cell wall damage that primes immunity in Arabidopsis. Disruption of cell wall integrity by inhibition of cellulose biosynthesis promotes pattern-triggered immunity transcriptionally in a manner dependent on the receptor kinase MALE DISCOVERER 1-INTERACTING RECEPTOR LIKE KINASE 2 (MIK2). Notably, while MIK2 can perceive peptides of the large SERINE RICH ENDOGENOUS PEPTIDE family, a single member of this family is transcriptionally induced upon cell wall damage and is required for subsequent responses such as lignin lignification and immunity priming. Collectively, our results identify a specific SCOOP-MIK2 ligand-receptor module as an important surveillance system, connecting plant cell wall integrity sensing with immunity.

Endogenous RALF peptide function is required for powdery mildew host colonization

Henriette Leicher *, Sebastian Schade, Ralph Hückelhoven, Martin Stegmann

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.
In Arabidopsis, the receptor kinase FERONIA (FER) was found to be a powdery mildew susceptibility factor 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 genetic approach, we now show that RALF peptides are required for successful powdery mildew infection on Arabidopsis. Loss of multiple leaf-expressed RALF peptides confers enhanced powdery mildew resistance, similar to loss of FER. We show that enhanced resistance in fer and ralf mutants is independent of defense hormone signalling. Surprisingly, we provide evidence that RALF-mediated powdery mildew susceptibility is partially independent of FER. We hypothesize that powdery mildew fungi require RALF-mediated apoplastic pH modulation for successful infection.

Connecting Membrane Receptor Signalling And Effector-Triggered Immunity Via Helper NLR Phosphorylation

Renzo Villena-Gaspar, Sophie Johnson, Paul Derbyshire, Jonathan DG Jones And **Frank LH Menke***

Pattern recognition receptor (PRR) signalling and effector-triggered immunity (ETI) are two stages of plant immunity and are connected through mutual potentiation (Ngou et al. 2021). While the role of phosphorylation in PRR signalling has been widely demonstrated, how phosphorylation modulates ETI is much less clear. ETI is based on the activation of nucleotide-binding leucine rich repeat proteins (NLRs) that come in three flavours, Toll interleukin receptor-like (TIR) NLRs, coiled-coil (CC) NLRs and CC-RPW8-like NLRs. NLRs get activated via direct or indirect detection of pathogen-derived effectors and can act in pairs or as part of a network with sensor and helper NLRs. TIR NLR activation requires EDS1-SAG101-NRG1 complex to trigger HR and and the EDS1-PAD4-ADR1 complex for resistance. N requirement gene 1 (NRG1) is an CC-RPW8-like NLR that functions as a helper NLR and upon TIR activation likely assembles into a membrane located higher-order complex or resistosome, functioning like a calcium channel. NRG1 resistosome formation requires both PTI and ETI (Feehan et al. 2023) but how PTI contributes to resistosome formation is not known. We have analysed the phosphorylation of NRG1 in response to PTI, and PTI plus ETI and used Phos-Tag gels to show that PTI induces phosphorylation. We identified and quantified the phosphorylated residues by LC-MS using by label free quantification. Two residues in the N-terminal portion of NRG1 are necessary and sufficient for HR in transient assays in N.benthamiana. Our results show that phosphorylation of helper NLR NRG1 in response to PRR activation is required for ETI, revealing a molecular mechanism for PTI/ ETI mutual potentiation.

References

Feehan JM, Wang J, Sun X, Choi J, Ahn HK, Ngou BPM, Parker JE and Jones, JDG. 2023. Proc Natl Acad Sci U S A 120, e2210406120.
Ngou BPM, Ahn HK, Ding P and Jones JDG. 2021. Nature 592, 110-115.

Evolutionary insights into the CTNIP-HSL3 Signaling Module Across Angiosperms

Oliver Johanndrees*, Jack Rhodes, Cyril Zipfel

Plants harbor a myriad of secreted signaling peptides; yet, only a few are functionally defined ligand-receptor pairs. Within these, experimentally validated ligand-receptor interaction sites are even fewer. Recent studies identified the CTNIP (also called SCREW) family of stress-responsive secreted peptides and their receptor, HSL3 (also called NUT), a leucine-rich repeat receptor kinase. The CTNIP-HSL3 signaling module is conserved across Angiosperms and is involved in both development and immunity. Notably, CTNIP recognition is con-specific, raising questions about the underlying evolutionary and biochemical mechanisms. We thus aimed to elucidate the ligand-binding mechanism of CTNIPs by identifying conserved binding residues across HSL3 homologs. Additionally, we investigated potential CTNIP residues that contribute to con-specific recognition through mutational analyses. We conducted phylogenetic analyses to examine the conservation and divergence of HSL3 homologs among various plant families. AlphaFold3-based protein predictions of HSL3 with CTNIP ligands guided our mutational assays. Functional assays and ligand-binding studies were performed to assess interaction specifics between CTNIP peptides and HSL3 homologs from diverse plant species. Our research identified an active CTNIP-HSL3 signaling module in barley, marking the first identification of functional CTNIPs in Monocots and allowing more broad-scale comparisons. Ligand-binding studies highlighted conserved residues crucial for binding across Angiosperm HSL3 homologs, suggesting a shared interaction mechanism. Through a combination of phylogenetic analysis, structural modeling, and experimental validation, this study provides important insights into the conserved binding mechanisms of the CTNIP-HSL3 signaling module. It identifies key residues critical for ligand binding and con-specificity, laying the groundwork for exploring peptide-receptor co-evolution and developing novel ligand-binding specificities in plant receptor kinases.

Overlapping and distinct recognition specificity for exogenous and endogenous signalling peptides in MIK2-family receptors from different plant lineages

Ralph Hückelhoven*, Julian Maroschek

Fusarium spp. cause severe economic damage in many species of cultivated plants exemplified by Fusarium Head Blight or Panama Disease. Microbe-associated molecular patterns (MAMPs) can be perceived by plants supporting disease resistance via the activation of pattern-triggered immunity (PTI). However, knowledge of MAMPs or corresponding plant immunity components is largely lacking for Fusarium spp.. We identified a new peptide elicitor fraction present in Fusarium and related fungal species, which elicits PTI responses in monocots and dicots. We mapped the causal mutation in an elicitor-insensitive Arabidopsis thaliana mutant (fere1) to a leucine-rich receptor-like kinase (MIK2). PTI loss-of-function in fere1 was fully complemented with the full-length FERE1/MIK2 protein. The strength of the phenotype in fere1 and independent mik2 mutants supports that MIK2 is a new key component in sensing Fusarium. MIK2 also contributes towards basal resistance to Fusarium wilt. Genetic interaction studies suggest a canonical PTI signaling model for Fusarium elicitor functions. We now widened MIK2 functions to the perception of comparable elicitors from a broader spectrum of fungal species. Genetic interaction of MIK2 with PTI signalling components and new data on receptor-elicitor-interaction further establish MIK2 as a probable pattern-recognition receptor. Because MIK2 was also described as a receptor for endogenous SCOOP peptides that act as immune-stimulating phytocytokines in Brassicaceae, we studied MIK2-family receptors from plant families that do not encode SCOOP peptides. Data suggest cross-family conserved MIK2 function in PTI but unique functions in phytocytokine signaling in Brassicaceae. Data support that MIK2 receptor like kinases evolved as pattern recognition receptor and subfunctionalized, specifically in Brassicaceae, for SCOOP peptide recognition.

Session II: Reproduction

Floral innovation in the Asteraceae evolved through modifications in plant stem cell peptide signaling

Daniel L. Jones, Reid Selby, Andrew C. Willoughby, Emily L. Yaklich, Pedro Jimenez Sandoval, Vandana Gurung, Anna T. DiBattista, Jakub Baczynski, Ashley D. Crook, Andra-Octavia Roman, Feng Wang, Teng Zhang, Riley Schuld, Jennifer R. Mandel, Paula Elomaa, John M. Burke, Julia Santiago, **Zachary L. Nimchuk***

How evolution acts to generate novel developmental forms is a key question in biology. Flowering shoot (inflorescence) architecture varies significantly across plant families, is linked to many domesticated crop traits, and is considered a target for future genetic engineering. The Asteraceae, which includes sunflowers and daisies, is the largest flowering plant family on the planet. Their success is linked to the evolution of the capitulum, a novel inflorescence form that evolved to mimic the appearance of a single flower for the purpose of attracting pollinators. During capitulum development, the shoot stem cell population undergoes prolonged expansion in many Asteraceae which facilitates the formation of multiple whorls of flowers (florets). Here, we show that capitulum evolution in the Asteraceae paralleled the degradation of CLAVATA3 peptide (CLV3p) signaling, which plays a key role in repressing shoot stem cell proliferation in all Angiosperm species studied to date. Using expanded genomic resources, we find that the Asteraceae evolved a unique CLV3p variant that displays reduced stem cell repressing activity in Asteraceae and other flowering plants. This loss of activity is associated with reduction in the repression of WUSCHEL, a key downstream target of CLV3p signaling in Angiosperms. We traced this reduction in activity to specific CLV3p residues and show that these impair binding to CLV3p receptors. Using genetically tractable Asteraceae models we show that reversion to fully active CLV3p impairs capitula and floret development. Lastly, we trace the evolution of CLV3 and CLV3p receptors from the Asteraceae into key outgroups drawing inferences in the evolution of the capitulum form. Collectively, our work demonstrates that the evolutionary novelty in Asteraceae shoot inflorescence architecture was driven by reductions in CLV3p signaling, which enabled the relaxed stem cell proliferation necessary to generate the capitulum structure. Our results further suggest that this pathway could be exploited to alter Asteraceae form.

Beyond receptors – Regulation of RhoGTPase signaling during pollen germination

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Polar growth is crucial for many cellular and developmental processes. It requires the polar accumulation of growth factors at a specific site within the cell. Polar growth is often regulated by receptor kinase signalling, which senses external cues. However, how the perceived signals are transduced into the cell to activate the cellular responses leading to polar accumulation of proteins is still not understood. We use pollen germination as a model to study how this dormant cell establishes a de novo polar growth domain after cell activation. The initiated polar growth requires multiple feedback loops to balance cellular expansion and stabilising mechanisms. The RhoGTPases RHO OF PLANTS (ROPs) are central molecular switches that integrate these feedback loops. ROP activity is, amongst others, regulated by activating ROP GUANINE NUCLEOTIDE EXCHANGE FACTORS (ROPGEFs). Even though multiple ROPGEFs are present in pollen grains, we show that specific ROPGEFs are crucial for pollen germination and establishing a polar protein domain at the plasma membrane. Further, we show that a kinase cascade of PDKs and AGCVIII kinases restricts pollen germination by phosphorylating multiple proteins required for pollen germination. Using phosphoproteomics, we identified novel AGCVIII kinase-dependent phosphorylation sites of ROPGEFs that restrict their activity and thus prevent premature pollen germination.

On the role of stem cell regulators in shaping barley spike architecture

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Grasses exhibit a wide variety of inflorescence architectures, from the complex branched inflorescences of the Oryzeae tribe (rice), where grains develop on primary and secondary branches, to simple spike-type inflorescences of the Triticeae tribe (e.g. barley and wheat), where grains develop on short vestigial axes called rachillae. The inflorescence architecture depends on shape, longevity, and determinacy of meristems that direct the growth of the main rachis and lateral branches. However, how individual meristem activities are determined and integrated within complex inflorescences is not yet understood. Here, we found that the activity of distinct meristems in the barley inflorescence is coordinated by a signalling pathway comprising the receptor-like kinase Hordeum vulgare CLAVATA1 (HvCLV1) and the secreted CLAVATA3/ENDOSPERM SURROUNDING REGION (CLE)-family peptide FON2-LIKE CLE PROTEIN1 (HvFCP1). HvFCP1 interacts with HvCLV1 to promote spikelet formation but restricts inflorescence meristem and rachilla meristem proliferation. Hvfcp1 or Hvclv1 mutants generated branched inflorescences with additional rows of spikelets and supernumerary florets. Transcriptome analysis suggested that HvFCP1/HvCLV1 signalling controls inflorescence branching through the regulation of trehalose-6-phosphate synthesis and sugar transport.

Insights from previous studies in Arabidopsis Thaliana suggested a possible compensatory effect on the Hvclv1 inflorescence phenotype by members of the closely related Hordeum vulgare BARELY ANY MERISTEM (HvBAM) gene family. Here, we investigated the function of two additional receptor-like kinases, HvBAM1 and HvBAM2, and their genetic interaction with HvCLV1 by the generation of higher-order mutants. While the single Hvbam mutants only slightly affected the morphology of the barley spike, mutant combinations with Hvclv1 displayed branches and multi-floret spikelets. Transcriptome analysis of mutant combinations, using bulk and single-cell RNA sequencing revealed perturbations in multiple pathways, involving genes that regulate cell division, auxin signalling, trehalose-6-phosphate metabolism, and sucrose synthesis. With this study, we uncovered the role of CLAVATA receptors in the regulation of different meristem types comprising the barley spike and demonstrated the potential to engineer inflorescence architecture through the specific regulation of meristem activities.

RALF/LRX implication in cell wall integrity during tomato fruit formation

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Cell wall is one of the key determinants of plant cell and organ shape. Progression in ripening induces the degradation of cell wall components, where the pectin degradation is the main factor, and this leads to softening of the fruit. This process has to be well regulated to ensure that the integrity of cell wall and the fruit growth are well coordinated. Small Signaling Peptides from Rapid Alkalinization Factor (RALF) family are emerging as key signaling components for cell wall integrity during cell wall remodeling. RALFs are cys-rich peptides conserved in terrestrial plants. RALFs peptides have been recently reported to have a dual role. They can bind Leucine-Rich Repeat Extensin proteins (LRXs) to have a structural role, and they can bind to Catharanthus roseus RLK1-Like (CrRLK1L) receptors in association with LORELEI (LRE)-LIKE GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORED PROTEIN (LLG) forming a trimeric signaling complex. A tomato fruit formation is a perfect model to further understand the role of this mechanism, since requires a tightly regulated expansion of the cell wall during the rapid cell elongation phase, and during the late cell wall softening phase. We identified SlRALF27/33/35 that are highly expressed in the tomato fruit at green stage. Biochemical characterization showed that the three RALFs interact with two SlLRX2/5. However, only SlRALF33/35 and SlLRX5 expression continues at tomato fruit red stage. Physiological characterization demonstrated that SlRALF33/35, but not SlRALF27, induce the typical RALF responses, such as root growth inhibition, ROS production and pH alkalinization of the media. We are in the process of generating CRISPR mutant tomato lines with the objective to further understand the role of RALFs/LRXs complex during tomato fruit maturation.

Antagonistic CLE peptide pathways shape root meristem tissue patterning

Hang Zhang, Qian Wang, Noel Blanco-Touriñán, **Christian S. Hardtke***

Secreted CLAVATA3/EMBRYO SURROUNDING REGION (CLE) peptide ligands dimension the stem cell niche of Arabidopsis shoot meristems by signaling through redundant and cross-compensating CLAVATA1 (CLV1)-type receptor kinases. In the root meristem, the CLV1 homologs BARELY ANY MERISTEM 1 (BAM1) and BAM2 drive CLE13/16-mediated formative divisions that produce the ground tissue layers. We found that BAM1/2 are also required to initiate the vascular phloem lineage and that cross-compensation and redundancy between CLV1-type receptors as observed in the shoot does not operate similarly in the root. Rather, BAM3-mediated CLE45 signaling antagonizes BAM1/2-mediated CLE11/12/13 signaling in the phloem initials (but not in the ground tissue). We further observe spatiotemporally contrasting CLE signaling requirements for phloem initiation and differentiation, which are shaped by congruence with the SHORT ROOT pathway. Thus, quantitatively graded CLE signaling through different peptide-receptor pairs is required to initiate the phloem lineage and guide its differentiation. Our findings suggest an intricate quantitative interplay between distinct and antagonistic CLE signaling pathways that organizes tissue layer formation in the Arabidopsis root meristem.

Session III: Stress signalling and mechanosensing

PBLs as Novel Players in Single-Cell Plant Signaling Specificity

Irene Guzmán-Benito*, Niko Geldner

Plants sense and integrate different environmental inputs and maintain their identity during signal transduction to prompt diverse cellular responses. The mechanisms responsible for signaling specificity are poorly understood and have only been studied in whole plants, organs, or artificial systems.

The endodermis is a cell layer that establishes the main extracellular diffusion barrier. Our study takes a novel approach, using the Arabidopsis root endodermis as a unique in planta cellular model system to stimulate two distinct signaling pathways in precisely the same cell type. This model provides a straightforward way to test and understand the specific signaling mechanisms within individual plant cells.

We focus on 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, a large class of proteins involved in the early stages of signal detection, are potential critical nodes for determining cellular signaling specificity.

Our findings indicate that FLS2 signaling in the endodermis does not require the highly abundant SGN1 PBL kinase, which is strongly needed for SGN3 signaling, but instead relies on BIK1, despite its basal low expression in this tissue. We show that BIK1 discerns between activated SGN3 and FLS2 receptors, even when expressed to higher levels under the endodermis-specific SGN1 promoter. Moreover, both SGN1 and BIK1 localize to the plasma membrane. While SGN1 localizes polarly to the cortex-facing plasma membrane domain, BIK1 localizes to the whole plasma membrane. Overall, we demonstrate that SGN1 and BIK1 show an outstanding capacity to specifically transduce signals of SGN3 and FLS2, respectively, and suggest particular requirements for different PBL signaling interactions. To investigate these conditions further, we conduct CRISPR knock-outs of all endodermally–expressed PBLs to identify the complete set of PBLs required for SGN3 or FLS2 signaling in the endodermis. This is combined with generating readouts for rapidly activated plasma membrane-localized targets of PBLs. In addition, to understand the role of each domain in PBL interaction mechanistically, we run a chimeric domain-combination analysis.

Our ultimate objective is to unravel the molecular foundation of LRR–RLK signaling specificity at a single-cell level.

Phytosulfokines as rapid cell wall mediators during root growth in Arabidopsis thaliana

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A multitude of phytohormones regulate cell division and elongation during root growth. Phytosulfokines (PSKs) are plant peptide hormones which contribute to root elongation as well as root meristem maintenance and xylem differentiation. PSKs are primarily recognized by Phytosulfokine Receptor 1 (PSKR1), which localizes to the plasma membrane (PM). Although the composition of the PSKR1 signaling complex has been the main target of recent research, the exact mechanisms of PSK-signaling during root growth remain largely unknown. Therefore, understanding the organization and output of this receptor complex in the PM is of great interest.

Single-particle-tracking Photoactivated Localization Microscopy (sptPALM) is a powerful superresolution technique to study dynamic properties of individual receptors in the PM in vivo. Tracking proteins via sptPALM gives access to their diffusion coefficient as well as the properties of nanosized clusters in the PM. Their composition and organization in the PM are assumed to be closely related to signaling specificity and integration. Investigation of these parameters in dependence of hormone application and genetic background provides new insights into hormone perception within the dynamic environment of the PM.
We found that the spatiotemporal behavior of PSKR1 observed by sptPALM is rapidly altered during hormone perception. Moreover, this fast PSK-dependent response is dependent on the state of the cell wall. The Arabidopsis thaliana (A. thaliana) line comfortably numb 2 (cnu2) encompasses overexpression of a pectin-methylesterase-inhibitor in the background of the receptor-like-protein 44 (RLP44) loss-of-function mutant. The mobility and cluster formation of PSKR1 deviate in the cnu2 background, which indicates a profound role of the cell wall status during early PSK sensing.
To understand the interaction of PSK perception and cell wall properties, we performed Atomic Force Microscopy (AFM) measurements of A. thaliana hypocotyl cells during hormone treatments. Our experiments reveal that PSK triggers a rapid softening of the cell wall in contrast to other root growth promoting phytohormones such as brassinolide. These results suggest that PSKR1 acts as part of a cell-wall-sensitive signaling complex with distinct functions. Simultaneous sptPALM observations of PSKR1 with other cell wall integrity mediators such as RLP44 will further clarify the interplay of the PSKR1 receptor complex and cell wall status. The integration of cell physiological response and single molecule dynamics could provide a refined understanding of PSK-signaling and new links between PSK and the cell wall, adding a valuable parameter to existing models of the elongating root.

Regulated Cleavage and Translocation of FERONIA Control Immunity in Arabidopsis Roots

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Plant roots exhibit localized immunity (LI) mainly in the transition (TZ), and elongation zones (EZ). Plasma membrane (PM)-localized receptor-like kinases (RLKs) can mediate the plant's response to rhizosphere bacteria. However, how RLKs are involved in triggering LI in roots remains unclear. Here, we identified dual actions for the RLK FERONIA (FER) in the LI response of Arabidopsis (Arabidopsis thaliana). The FER cytoplasmic domain is cleaved and translocated to the nucleus (FERN) to activate LI in the TZ and EZ in response to colonization by beneficial and pathogenic bacteria. In the absence or cessation of bacterial infection, full-length FER is PM-localized to maintain growth. Upon colonization and invasion by a high titer of bacteria, mature RAPID ALKALINIZATION FACTOR23 (RALF23) peptide accumulates and activates the matrix metalloproteinase At2-MMP, which triggers FER cytoplasmic domain cleavage specifically in the TZ and EZ to activate LI. This work demonstrates that two molecular forms of a single RLK balance growth and immunity via LI activation in Arabidopsis roots.

Rapid Alkalinization Factor 22 (RALF22) has a structural and signalling role in Arabidopsis root hair cell wall assembly

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Secreted Rapid ALkalinization Factor (RALF) peptides have emerged as key components controlling cell wall integrity. However, the inner workings of the RALF pathway remain enigmatic. Here we show that RALF22, a root hair expressed RALF, has a dual signaling and structural role during cell growth. RALF22 loss-of-function root hairs are short and frequently burst due to loss of wall integrity. Exogenous RALF22 treatment induces a FERONIA-dependent root hair growth arrest and a FER-independent increase in cell wall porosity. Our data show that this duality is the result of RALF22 interacting with (1) the LLG1/FER transmembrane receptor complex to regulate downstream signaling, including intracellular calcium-dynamics, and (2) the integral cell wall proteins LRX1 and LRX2 to regulate pectic cell wall assembly. In the root hair cell wall, RALF22 forms periodic circumferential rings which colocalize with rings of block-wise demethylated homogalacturonan (HG) and LRX1. Polycationic RALF22 and RALF22-LRX1 bind to and induce the condensation of polyanionic HG in a charge dependent manner. In vivo, the LLG1-RALF22-FER and RALF22-LRX1-pectin interactions are mutually exclusive. We propose a new mechanism in which RALF22 simultaneously regulates periodic pectin assembly through LRX1/2 and cell wall sensing through LLG1/FER.

Exploring the role of GHR1 in plant immunity: insights from Arabidopsis mutants with constitutively open stomata

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Stomata regulate gas exchange and transpiration in plants, but they can also serve as gateways for foliar pathogens like Pseudomonas syringae pv. tomato (Pst). Stomatal pores are flanked by guard cells that close the stomata to prevent pathogen entry. However, extended stomatal closure can hinder plant resistance. Pst exploits regulative networks in guard cells, e.g., by secreting the toxin coronatine to reopen closed stomata, allowing bacterial entry. At post-invasive stages, Pst induces stomatal closure by hijacking ABA-signalling to enhance apoplastic hydration. Increased apoplastic hydration creates favourable conditions for bacterial proliferation, observed as water-soaked lesions on the leaves.

In previous literature, pathogen-triggered stomatal closure, or stomatal immunity, has been regarded as one of the resistance-determining steps in Pst pathogenesis. However, recent research highlights a secondary immune response in which stomata reopen at later infection stages to prevent excessive apoplastic hydration, coined as water immunity. To systematically evaluate the relative importance of stomatal versus water immunity, we selected a diverse set of Arabidopsis mutants with stomatal dysfunction phenotypes and performed infection assays using Pst pv. tomato DC3000. Spray-inoculated plants with disrupted stomatal closure were significantly more resistant against Pst and exhibited less water soaking compared to Col-0. Our results highlight the importance of open stomata at the late stages of defense against Pst, suggesting that initial stomatal immunity is not the determining factor in disease resistance.

One of the mutants that showed significantly increased resistance to Pst despite its persistently open stomata was ghr1-3, which lacks the leucine-rich receptor-like pseudokinase GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1 (GHR1). Our current research investigates the role of GHR1 in stomatal regulation using reverse genetic and proteomic approaches. This includes characterizing immune responses and identifying novel molecular interactions under biotic stress. While the role of GHR1 in abiotic stress responses is relatively well-understood, its molecular mechanisms in plant immunity remain largely unexplored.

Poltergeist-Like 2 (PLL2)-dependent activation of the wound response distinguishes systemin from other immune signaling pathways

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Systemin was identified in 1991 as the first plant signaling peptide, and is required for defense against insect herbivores in tomato plants. Systemin was first perceived as a hormone-like, long-distance messenger mediating the activation of systemic defense responses far from the site of insect attack. It was later shown to rather act as a phytocytokine, amplifying the local wound response for production of downstream signals resulting in defense gene activation in distal tissues. Systemin perception and signaling rely on the systemin receptor SYR1. However, SYR1-dependent signaling, and how systemin signaling differs from other phytocytokine signaling pathways, is largely unknown. Here we report that systemin activates the poltergeist-like phosphatase PLL2 in a SYR1-dependent manner. In contrast to the PLL-mediated inhibition of pattern-triggered immunity, PLL2 activates early systemin responses at the plasma membrane including the rapid inactivation of plasma membrane proton pumps by dephosphorylation of their regulatory C-termini, as well as downstream defense gene expression and, ultimately, insect resistance.

Session IV: Complex formation and technical advancement

Redox-dependent extracellular interaction networks of Cysteine-rich Receptor-Like Kinases

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Cellular processes in plants are tightly regulated by extracellular Receptor Kinases (RKs), which play a crucial role in perceiving and relaying signals for stress responses and developmental processes. An intriguing aspect of RK activation is the generation of extracellular Reactive Oxygen Species (ROS), which function as signalling molecules to amplify and relay stress or developmental signals. Furthermore, specific cellular organelles with differential permeability toward these reactive species add complexity to the spatial and temporal regulation of ROS-mediated signalling. The presented work explores the modulatory role of ROS on the interaction networks of the Cysteine-Rich Receptor-Like Kinases (CRKs) by generating a large redox-dependent interactome assay (RIACRK) between ectodomains of CRKs. Parallelly, a map of oxidative modifications of the CRK extracellular domains (ECDs) was generated. These findings suggest that ROS modulate CRK interactions through direct chemical modifications on the cysteine residues of CRK ECDs. Multilayer filtering of the RIACRK networks by CRK expression pattern, oxidative modification signature and ROS modulation of interactions facilitates candidate selection. Using this approach and the available literature, CRK28 was selected for further in planta validation studies. Combining physiological measurements, proteomics and phosphoproteomics studies, revealed a strong association between CRK28 and processes with elevated ROS production such as senescence and defence responses. This study sheds light on the intricate interplay between RKs, ROS signalling, and CRK-mediated cellular responses, offering new insights into the regulatory mechanisms governing plant stress responses and developmental processes.

Peptide REF1 is a local wound signal promoting plant regeneration

Chuanyou Li*, Wentao Yang, Huawei Zhai

Plants frequently encounter wounding and have evolved an extraordinary regenerative capacity to heal the wounds. However, the wound signal that triggers regenerative responses has not been identified. Here,through characterization of a tomato mutant defective in both wound-induced defense and regeneration, we demonstrate that in tomato, a plant elicitor peptide (Pep), REGENERATION FACTOR1 (REF1), acts as a systemin-independent local wound signal that primarily regulates local defense responses and regenerative responses in response to wounding. We further identified PEPR1/2 ORTHOLOG RECEPTOR-LIKE KINASE1 (PORK1) as the receptor perceiving REF1 signal for plant regeneration. REF1-PORK1-mediated signaling promotes regeneration via activating WOUND-INDUCED DEDIFFERENTIATION 1 (WIND1), a master regu lator of wound-induced cellular reprogramming in plants. Thus, REF1-PORK1 signaling represents a conserved phytocytokine pathway to initiate, amplify, and stabilize a signaling cascade that orchestrates wound-triggered organ regeneration. Application of REF1 provides a simple method to boost the regenera tion and transformation efficiency of recalcitrant crops.

QSK1 modulates ER-PM contact sites to regulate small molecules transport through PD

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Plasmodesmata (PD) act as channels that facilitate the diffusion of small molecules between adjacent cells, playing a vital role in maintaining plant growth by ensuring intercellular connectivity at the tissue level. During both growth and in response to external environmental stimuli, cells may experience osmolarity changes. In such cases, plasmodesmata adjust their permeability to maintain cellular osmolarity by either loosening or restricting their apertures to regulate the movement of essential molecules. This regulatory process is tissue-type dependent and influenced by the source-sink relationship within the plant. QSK1 ('Qian Shou' kinase 1), a receptor-like kinase from the LRRIII subfamily, is dual-localized at the plasma membrane and in PD. It becomes enriched in PD in response to osmolarity changes, a process linked to its phosphorylation status. However, the mechanisms by which QSK1 phosphorylation influences PD dynamics across tissues remain unclear.

We conducted spatiotemporal plasmodesmata proteomic analysis in various tissues of Arabidopsis and performed in vivo interactome analyses of QSK1 using affinity enrichment mass spectrometry. Through data mining and unsupervised learning, we identified a QSK1-centered protein complex associated with mechanical sensing that responds to source-sink interactions. We propose that modulation of QSK1 phosphorylation affects enrichment of ER-PM contact proteins at plasmodesmata, and thereby also affects the symplastic transport of small molecules through plasmodesmata.

Mechanosensing and Signalling: The interplay of FERONIA, pectin, and RALF peptides in plant cell walls

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The receptor-like kinase FERONIA (FER) is a central hub for sensing external signals, including the cell wall status. Acting as a mechano-sensor, FER transduces mechanical signals from the plant cell wall and regulates cellular expansion rates. On the other hand, FER functions as a peptide hormone receptor, with known ligands being members of the RAPID ALKALINIZATION FACTOR (RALF) family. However, it is still not completely understood how cell wall signals are molecularly integrated and what the exact nature of the signal sensed by FER in the cell wall is.

Pectin, a polysaccharide crucial for cell wall structural integrity, is deposited to the apoplast in a highly methylesterified form and can be demethylesterified, thus revealing a negative charge on the remaining carboxyl groups. Our recent findings uncover that FER-dependent perception of RALF1 requires pectin modification (Rößling et al., 2024). Demethylesterified pectin can interact with RALF1 through charge-based interactions since RALF1 has a positive charge, adding a critical connection between the demethylesterification machinery of the cell wall component pectin and the extracellular peptide hormone family. Furthermore, our data demonstrate that both pharmacological and genetic interference with the methylation status of pectin, disrupts RALF1 signalling, emphasising the necessity of demethylesterified pectin for RALF1 binding. Interestingly, this mechanism operates independently of FER's extracellular matrix sensing through LEUCINE-RICH REPEAT EXTENSIN (LRX) proteins.

Our research highlights the integrative role of the extracellular matrix in plant cellular responses, particularly in extracellular ligand signalling. We propose that pectin's methylestrification status functions as a signalling scaffold for RALF peptides, linking extracellular matrix dynamics to peptide hormone-mediated responses and providing new insights into FER-dependent signalling pathways.

Immune signalling by cell surface-localised receptor-like proteins (RLPs) reveals its secrets

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The interaction between the apoplastic pathogenic fungus Fulvia fulva (formerly known as Cladosporium fulvum) and tomato, has been subject of study for various decades. Next to the Cf resistance genes, most of which were identified by the research team of Jonathan Jones (The Sainsbury Laboratory, Norwich, UK), our team has identified the matching effectors that are secreted by F. fulva. Cf proteins are receptor-like proteins (RLPs), which are cell-surface receptors that stand at the basis of the first layer of the plant innate immune system. During gene-for-gene co-evolution, F. fulva has evaded recognition by Cf proteins through mutation of its matching effectors. We found that the receptor-like kinases SOBIR1 and BAK1 are the regulatory co-receptors of RLPs and tomato Cf-4, which detects the Avr4 effector secreted by F. fulva, also requires these co-receptors to mediate resistance against this fungus. We have established (i) reactive oxygen species (ROS) burst, (ii) MAPK activation and (iii) cell death activation as indicators of the strength of the immune output and have transformed the Cf-4 gene to the model plant Nicotiana benthamiana, in which the encoded protein is functional. Receptor-like cytoplasmic kinases (RLCKs) are well-recognized to act as the initial cytoplasmic transducers, bridging cell-surface receptor complexes with their downstream signalling partners. The family of RLCKs is extremely large, with more than 100 members in tomato and in N. benthamiana. We have knocked-out multiple genes belonging to different RLCK class VII subfamilies in N. benthamiana:Cf-4 and observed that members encoded by RLCK-VII-6, -7, and -8, differentially regulate the Avr4/Cf-4-triggered biphasic ROS burst. In addition, members of RLCK-VII-7 were found to play an essential role in the Avr4/Cf-4-triggered hypersensitive response (HR) resulting in cell death, and in resistance against the oomycete pathogen Phytophthora palmivora, which in N. benthamiana is mediated by the RLP RESPONSIVE TO ELICITINS (REL).

Session V: Symbiosis

Immune peptide receptor and symbiosis regulator drive mutualistic interactions with plant growth promoting bacteria in rice

Kanako Inoue, Masako Fuji, Masanao Sato, Shota Kido, Takumi Murakami, **Yusuke Saijo***

Plants accommodate and utilize root-inhabiting microbes for adaptation to adverse conditions, such as nutrient deficiency, but the mechanisms underlying the mutualistic interactions remain poorly understood. An important layer for infection control is conferred by immunogenic peptides called phytocytokines, including plant elicitor peptides (Peps) that trigger and amplify defense responses through the leucine-rich repeat receptor kinases PEPRs. Here, we show that immunogenic OsPep peptides promote seedling growth in the presence of seed-derived bacteria in rice. A screen for plant growth promoting (PGP) bacteria in OsPep-applied plants reveals a Sphingomonas bacterial isolate, which colonizes the root to promote rice growth in a manner dependent on OsPep receptor OsPEPR1. Rice growth promotion by the bacterium and OsPeps also requires Ca2+/calmodulin-dependent protein kinase (CCaMK), a central regulator of mycorrhizal symbiosis. Cell biological analyses indicate that the bacterium relies on OsPEPR1 and CCaMK in separate steps during root colonization. The results suggest that the immunity and symbiosis signaling regulators both promote mutualistic interactions with PGP bacteria in paddy rice, in which mycorrhizal symbiosis is not effectively established.

RAPID ALKALINIZATION FACTOR 22 regulates root hair growth in response to fungal ethylene emissions

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In nature, plants and microorganisms communicate with each other by exchanging different signaling compounds including volatile compounds (VCs), some of which have biostimulating properties. In Arabidopsis, VCs from the fungal phytopathogen Penicillium aurantiogriseum promote growth, photosynthesis and root hair (RH) proliferation and hyper-elongation through mechanisms involving ethylene, auxin and photosynthesis signaling. A striking alteration in the proteome of roots of fungal VC-treated plants involves strong up-regulation of RALF22. To investigate the possible role of RALF22 in the fungal VC-promoted RH changes, we examinated the RH responses of ralf22 and fer-4 plants , which are impaired in RALF22 and its receptor FERONIA, to VCs emitted by P. aurantiogriseum. Our findings revealed that these plants did not respond to VC-promoted RH elongation and proliferation. Unlike in WT roots, fungal VCs did not enhance the transcript levels of RALF22 in roots of fer-4 and ethylene- and auxin-insensitive mutants and those of well-known RH-related genes in ralf22 and fer-4 mutants. Moreover, roots of plants defective in photosynthetic responsiveness to VCs exhibited weak RALF22 expression and RH growth responses to fungal VCs. Finally, we identified ethylene as the bioactive fungal VC, as VCs of ΔefeA strains of P. aurantiogriseum cultures impaired in ethylene synthesis weakly promoted RH proliferation and elongation in exposed plants. Collectively, our results demonstrate that RALF22 and FERONIA are determinant of the ethylene, auxin and photosynthesis signaling-mediated RH response to fungal ethylene emissions.

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Balancing nodule number with a CLV1-like receptor: identification of signalling proteins using a proteomics approach

Emma Guillierme *, Afroditi Katsaouni, Sylwia Struk, Kris Gevaert, Sofie Goormachtig

Soybean, an important crop for food, feed and oil production, can establish symbiotic interactions with rhizobia, which are nitrogen-fixing bacteria. The soybean-rhizobia interaction results in formation of nodules on plant roots, where fixed nitrogen provided by bacteria is exchanged for carbohydrates. For an optimal balance between benefits and costs of this interaction, plants tightly regulate their nodule number.
In soybean, rhizobia induce the expression of rhizobia-induced CLE (RIC) peptides which activate the autoregulation of nodulation (AON) pathway. These peptides are transported from root to shoot, where they are perceived by the soybean Nodule Autoregulation Receptor Kinase NARK. NARK is a homolog of Arabidopsis CLAVATA1 (CLV1), but it evolved independently and acquired a function in nodule regulation. Some knowledge about the downstream signalling could be transferred from CLV1 to NARK, such as the involvement of Kinase-Associated Protein Phosphatase (KAPP) 1/2. NARK signalling eventually results in secondary shoot-to-root signals to inhibit nodulation. For example, the downregulation of miR2111 allows root accumulation of Too Much Love (TML), a nodulation-inhibiting F-box protein. Limited knowledge is available about the downstream NARK pathway, but signalling via phosphorylation is expected based on the function of NARK. To fill this research gap, we performed phosphoproteomics and shotgun proteomics in soybean leaves. Three time points were selected for phosphoproteomics, based on gene expression of RIC in roots and miR2111 in leaves: 0, 3 and 7 days post-inoculation. Based on the first proteomics data, three candidate proteins were selected for further validation: a MAP kinase, a LRR receptor-like kinase and a GPI-anchored protein. This time series phosphoproteomics will allow the elucidation of dynamic phosphorylation changes and molecular interactions upon induction of NARK signalling, identifying downstream signalling proteins involved in AON. Further progress in this research will be discussed.

Receptor kinase dependent G-protein phosphorylation and its role in regulating signaling during soybean nodulation

Sona Pandey*, Swarup Roy Choudhury

Molecular inter-species dialogue between leguminous plants and nitrogen-fixing rhizobia results in the development of symbiotic root nodules. This is initiated by several nodulation-related receptors present on the surface of root hair epidermal cells. We have shown previously that specific subunits of heterotrimeric G proteins and their regulatory RGS (regulator of G-protein signaling) proteins act as molecular links between the receptors and downstream components during nodule formation in soybeans. Nod factor receptor 1 (NFR1) interacts with and phosphorylates RGS proteins to affect its biochemistry and regulate the G-protein cycle. Symbiosis receptor-like kinase (SymRK) phosphorylates Gα to make it inactive, and unavailable for Gβγ. We now show that like NFR1, SymRK also interacts with the RGS proteins to phosphorylate them. Phosphorylated RGS has higher GTP accelerating activity, which favors conversion of active Gα to its inactive form. Phosphorylation of RGS proteins is physiologically relevant, as overexpression of a phospho-mimic version of RGS protein enhances nodule formation in soybean. These results reveal an intricate fine-tuning of the G-protein signaling during nodulation, where a negative regulator (Gα) is effectively deactivated by RGS due to the concerted efforts of several receptor proteins to ensure adequate nodulation.

Session VI: Development I

A plasma membrane pH-stat coordinates cell surface processes and root development.

Kaltra Xhelilaj*, Michelle von Arx, Aleksander Paranov, Davide D'Assero, David Bierman, Felix Klingelhuber, Nga Pham, Natalie Faiss, Paul Derbyshire, Frank Menke, Cyril Zipfel, Marja Timmermans, Julien Gronnier

The power of hydrogen (pH) modulates virtually all cell bioactivities and living organisms evolved mechanisms to tightly control it. In plants, variations in root cell surface pH correlates with developmental transitions. However, how and why apoplastic pH is dynamically regulated from cell to cell remains largely unclear. Here we show that the membrane scaffold proteins REMORINs inhibit the activity of the Arabidopsis plasma membrane-localized H+ ATPases (AHA) thereby promoting alkalinization of the apoplast. The regulation of pH by REMORINs modulate numerous cell surface processes. In this context, our data suggests REMORINs function as membrane effectors, dynamically trapping a positive regulator of AHA activity. Our findings unveil a molecular mechanism underlying a root cell surface pH optimum and define its role in coordinating cell surface processes and root development.

Progression in xylem maturation relies on a non-cell-autonomous peptide-dependent mechanism

Salves Cornelis, Samy Carbonnel, David Molina, Laura Ragni and **Ora Hazak***

Roots, possessing conducting tissues, played a crucial role in the evolution of land plants. This adaptation allowed plants to separate their photosynthetic organs, which grow toward the light source, from those that forage for water and nutrients in the soil. In Arabidopsis root, xylem tissues differentiate gradually, with outermost protoxylem cell files maturating first, then the outer metaxylem, and finally inner metaxylem cells becoming fully functional. This gradual maturation allows developmental plasticity and higher root elongation capacity, which are crucial for the root function in foraging soil. The key transcription factors initiating final xylem differentiation were identified in previous studies, whereas signals that define the timing of the final differentiation were not yet described. In this study, we focused on root-expressed CLE peptides and their roles in vascular tissue differentiation. First, we created a collection of 33 CLE gene reporters, we characterized their expression pattern and grouped them based on this. Then, we created higher order CLE mutants, that miss xylem-specific CLEs or phloem-specific CLEs. In addition, we created lines with inducible expression of these peptides in the root elongation area, to examine their effect on the xylem differentiation. The xylem sextuple mutant and phloem quadruple mutants were created and xylem maturation was examined. Our finding show that xylem-secreted peptides induce final differentiation in the neighboring xylem cells. We found, that phloem-born CLE peptides have even more dominant effect on xylem differentiation, providing with a novel, non-cell-autonomous peptide-dependent mechanism shaping xylem tissue. To identify a cognate receptor(s) involved in this regulation, we characterized CLE receptor single and higher-order mutants for their xylem differentiation. Our results show, that BAM receptors mediate this developmental response, which is strongly delayed in the loss-of-function mutant. Interestingly, the transcriptomics analysis of seedlings treated with xylem-specific peptide uncovered a specific, cell-wall remodeling response, which is an important step in inducing final maturation of this tissue. Next, we were wondering, if final maturation of primary xylem can affect the timing of the switch to secondary growth. Indeed, delayed maturation of primary xylem, resulted in later switch to secondary growth. In conclusion, we uncovered a new non-cell-autonomous mechanism, where CLE peptides produced in phloem pole and metaxylem cells induce gradual maturation of primary xylem and initiation of the secondary growth.

Plasma membrane alterations during plant immune response

Isha Doiphode, Inder Kiel, **Peter Grones***

Understanding how external signals are perceived at the plasma membrane (PM) and how they affect its structure is crucial for comprehending the PM's role during cellular immune responses. In nature, organisms must constantly interpret a multitude of environmental cues to adapt and thrive amidst various plant pathogens. The PM, the outermost cellular membrane, consists of a complex and dynamic network of lipids and proteins that forms the boundary between a cell's contents and its surroundings. Embedded within this membrane are pattern recognition receptors (PRRs), essential for sensing both external and internal signals and transmitting them into the cell to elicit appropriate responses. Among these receptors are PEP RECEPTOR 1 and 2 (PEPR1 and PEPR2), leucine-rich repeat receptor-like kinases that are key members of the PRR family, adept at recognizing endogenous PEP peptides. Upon signal perception, not only do the receptors undergo changes, but other proteins not directly related to the signal, such as PIN-FORMED2 (PIN2) and BRASSINOSTEROID INSENSITIVE1 (BRI1), also undergo degradation. However, the precise extent and reasoning for changes in PM content and structure during immune signal perception remain unclear. In our investigation of PM changes upon PEP perception, we identified that H+-ATPase (AHA2) also undergoes degradation, though in a different time-dependent manner compared to PIN2 and BRI1. Additionally, we assessed whether all PEP peptides can trigger the internalization of PM proteins. While this was true for BRI1, it was not observed for PIN2. These findings highlight a possible non-redundancy among the members of this endogenous, danger-associated peptide family, shedding light on the complex dynamics of PM protein modulation in response to immune signals.

Session VII: Development II

RGF peptides and their cognate receptors play a role in Phtheirospermum japonicum haustorium development

Maxwell R. Fishman*, Anne Greifenhagen, Takanori Wakatake, Anuphon Laohavisit, Ryoko Hiroyama, Ken Shirasu

Parasitic plants initiate rapid de novo organogenesis of their feeding structure called a haustorium upon contact with a host. In the model parasitic plant, Phtheirospermum japonicum, haustorium formation begins with the perception of host-derived haustorium inducing factors (HIFs), such as 2,6-dimethoxybenzoquinone (DMBQ), by CARD1-like receptors. DMBQ quickly induces Ca2+ signalling and a MAP kinase signalling cascade that is followed by the up regulation of haustorium specific genes like the YUCCA monooxygenase, YUC3. However, there is scant information on additional signalling components involved parasitic plant haustorium development. We found that three of the thirteen root meristem growth factor (RGF) peptides in P. japonicum, PjRGF1/2/5, are upregulated following treatment with DMBQ. RGF peptides and their cognate receptors (RGFRs/RGIs) have been identified in Arabidopsis thaliana as being involved in root apical meristem maintenance and regulators of lateral root elongation. Interestingly, treatment of P. japonicum with any of these three PjRGF peptides can induce the first developmental stage of haustoria, called prehaustoria. Spatial expression patterns showed that only PjRGF2 and PjRGF5 were specifically expressed in the developing haustorium, suggesting that these two PjRGF peptides play a greater role in haustorium development than PjRGF1. The P. japonicum genome encodes six RGFRs and expression of several PjRGFRs localize to the haustorium. Additionally, using PjRGFR receptor chimeras we found that PjRGF1/2/5 are perceived by several PjRGFRs. Lastly, using a CRISPR hairy-root gene editing system, we found that PjRGF2 and PjRGFR1 are involved in DMBQ-induced prehaustorium development. From these data, we conclude that parasitic plants may have coopted a plant peptide hormone-receptor interaction that is typically associated with root apical meristem maintenance and lateral root development during haustorium formation.

The brassinosteroid receptor gene BRI1 safeguards cell-autonomous brassinosteroid signaling across tissues

Noel Blanco-Touriñán*, Surbhi Rana, Trevor M. Nolan, Kunkun Li, Nemanja Vukašinović, Che-Wei Hsu, Eugenia Russinova, Christian S. Hardtke

Brassinosteroid signaling is essential for plant growth as exemplified by the dwarf phenotype of loss-of-function mutants in BRASSINOSTEROID INSENSITIVE 1 (BRI1), a ubiquitously expressed Arabidopsis brassinosteroid receptor gene. Complementation of brassinosteroid-blind triple receptor mutants by BRI1 expression with various tissue-specific promoters implied that local brassinosteroid signaling may instruct growth non-cell-autonomously. Here we performed such rescues with a panel of receptor variants and promoters, in combination with tissue-specific transgene knockouts. Our experiments demonstrate that brassinosteroid receptor expression in several tissues is necessary but not sufficient for rescue. Moreover, such rescues do not occur with a recoded BRI1 gene although it produces an identical and functional BRI1 protein. Thus, complementation with tissue-specific promoters requires the genuine BRI1 gene body sequence, because it confers ubiquitous expression of trace BRI1 receptor amounts that are sufficient to promote brassinosteroid-dependent root growth. Our data, therefore, argue for a largely cell-autonomous action of brassinosteroid receptors, although brassinosteroid itself may instruct growth non-cell-autonomously through its targeted biosynthesis, transport and distribution.

Posters

Poster N° 1 – Flash Talk (part 1)

Cell wall integrity and elicitor peptide signalling modulate phytoalexin-mediated pathogen defence in Arabidopsis

Richard Noi Morton*, Ahalya Rajendran, Lian Fleischberger and Timo Engelsdorf

The plant cell wall provides mechanical support to plant cells and plays an important role in responses to abiotic and biotic stress. This is achieved by innate mechanisms which monitor cell wall integrity (CWI) and trigger compensatory responses should this integrity be impaired. Available knowledge suggests that several plasma membrane-localized proteins are associated with CWI surveillance, of which the receptor kinase THESEUS1 (THE1) has been identified as a key CWI monitor in Arabidopsis1,2.
We investigated defence-related responses initiated by THE1-dependent CWI signalling and found an accumulation of the phytoalexin camalexin upon CWI impairment caused by cellulose biosynthesis inhibition (CBI). Camalexin contributes to resistance against fungal pathogens and its biosynthesis is induced by the transcription factor WRKY33, MAP kinases and the phytohormones ethylene and jasmonic acid (JA)3. We show that upon CBI treatment, both THE1 and WRKY33 are required for camalexin accumulation, while THE1 is not required for WRKY33 expression. RNAseq analysis indicated upregulation of several genes involved in JA biosynthesis and signalling. Most of these genes were suppressed after co-treatment with the plant elicitor peptide Pep3, which is consistent with Pep3-dependent suppression of CBI-induced JA accumulation. Furthermore, camalexin accumulation depended on intact JA biosynthesis and Pep3-induced suppression was lifted after exogenous JA treatment, indicating a central role for JA in regulating camalexin accumulation after CWI impairment. Similar to Pep3, pathogen-derived elicitors such as flg22 and chitohexose were able to suppress CBI-induced camalexin accumulation, indicating the presence of a general regulatory mechanism balancing CWI with pattern-triggered immunity. In agreement with a role of CWI surveillance in pathogen defence, THE1 loss-of-function mutants showed increased susceptibility to the cell wall-penetrating fungal pathogen Colletotrichum higginsianum, while accumulation of camalexin and the defence-related phytohormone salicylic acid was reduced.

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Poster N° 2 – Flash Talk (part 1)

New constructs for functional characterization of THESEUS1.

Steven Zwartkruis*, Wiebke Häger, Tereza Tichá, Vivien Klein, Martijn Vandegehuchte, Gregor Madej, Christine Ziegler, Thorsten Hamann

THESEUS1 (THE1) is a receptor-like kinase involved in cell wall integrity maintenance. Loss-of-function the1-1 mutants show an increase in abscisic acid (ABA) production in response to sorbitol-induced hyperosmotic stress, whereas the1-4 gain of function mutants displays increased jasmonic acid (JA) production in response to isoxaben-induced cell wall damage. THE1 therefore seems to be a negative regulator of ABA and a positive regulator of JA. While several interaction partners of THE1 have been identified, toxicity to E. coli of the full length THE1 hampers the generation of constructs necessary for functional studies of THE1. We made three fluorescent protein fusion constructs of mutated, non-toxic, THE1 variants. This includes one variant without the extracellular malectin-like domains, one without the kinase domain and a presumed kinase-dead full-length variant (K636E substitution). These variants will be used for GFP-trap experiments to identify novel interaction partners of THE1 as well as for intracellular localization studies. This will allow us to study how and where THE1 perceives stress and activates downstream responses. Taken together, we present new variants of THE1 for functional studies of THE1 and want to use these to reveal how THE1 can act simultaneously as positive regulator of JA and negative regulator of ABA.

Poster N° 3 – Flash Talk (part 1)

Ca2+ signaling in CLE45-regulated protophloem development

Kunkun Li, Christian S. Hardtke

Phloem is a major tissue responsible for transporting nutrients and various signaling molecules and thus plays pivotal roles in root growth and development. The temporal-spatial organization of cell division and differentiation determines cell growth and is required for proper tissue formation. Defects in the formation of the early, so-called protophloem typically lead to a short root phenotype in Arabidopsis thaliana. Such defects are typically linked to hyperactive CLAVATA3/EMBRYO SURROUNDING REGION-RELATED 45 (CLE45) peptide signaling. CLE45 is mainly perceived by the BARELY ANY MERISTEM 3 (BAM3) receptor to regulate protophloem differentiation. An apoplastic pH gradient is responsible for differential CLE45 perception along the spatio-temporal trajectory of protophloem development. Ca2+ is one of the most important second messengers in various abiotic and biotic stresses and is an essential nutrient element for plant growth. Whether Ca2+ is involved in protophloem development is unknown. Here we found a cytosolic calcium gradient ([Ca2+]cyt) in protophloem which correlates with the apoplastic pH gradient. Thus, Ca2+ may directly or indirectly influence CLE45 signaling. Here we found that external Ca2+ homeostasis can modulate CLE45-regulated protophloem differentiation. With increasing external CaCl2 concentration, CLE45 signaling is enhanced. The Ca2+-dependent enhancement is unique for CLE45 signaling and conveyed by the BAM1 and BAM3 receptors. Therefore, the distinct and dynamic ion environment in developing root protophloem may guide its differentiation.

Poster N° 4 – Flash Talk (part 1)

HPCAL1 – LRR receptor kinase coordinates nitrate response through NRT2.1 phosphorylation and H2O2 signaling.

Tatsiana Straub, Zhi Li, Waltraud Schulze

Nitrate is not only a major N source for the plants, but also a nutrient signal that triggers regulatory cascades affecting plant growth and development. Previously we identified a receptor kinase (HPCAL1, AT5G49770) as a pivotal regulator of the Arabidopsis NRT2.1 nitrate transporter. We propose that HPCAL1 acts as a molecular switch that toggles NRT2.1 between two active states. HPCAL1 interacts with the N-terminus of NRT2.1 when S28 is dephosphorylated, and it then phosphorylates S21 to activate NRT2.1. In addition to NRT2.1 phosphorylation we hypothesized that HPCAL1 is involved in sensing apoplastic H2O2 upon nitrate availability. Mutation in hpcal1 gene changes root growth and ROS accumulation across eH2O2 treatment under sufficient nitrate condition. Furthermore, we identify a PIP2 protein as another possible player in NO3--dependent ROS signaling. We found that nitrate and hydrogen peroxide stimulation increase C-terminal phosphorylation of PIP2 aquaporin at the same amino acid in WT but not in hpcal1 mutant. HPCAL1 may therefore represent a missing puzzle piece that links ROS signaling and plant response to the changing nitrate availability.

Poster N° 5 – Flash Talk (part 1)

Structural and Computational Investigation of LORE Receptor Binding Specificity to Medium Chain 3-Hydroxylated Fatty Acids

Fan-Yu Yu, Lin-Jie Shu, Antonella Di Pizio, Stefanie Ranf

LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION or SD1-29) is a pattern recognition receptor mediating the immune response upon sensing medium chain 3-hydroxylated fatty acids (mc-3-OH-FAs) in Brassicaceae [1]. The sensitivity of Arabidopsis thaliana LORE to mc-3-OH-FAs is specific to those with 8-12 carbon atoms [2]. As a member of the S-domain receptor kinase family, the extracellular domain (ECD) of LORE comprises two lectin-like domains, an epidermal growth factor-like domain and a plasminogen-apple-nematode domain. Based on ECD swapping with the 3-OH-FA non-binding ortholog, SD1-23 [3, 4], and molecular dynamic analysis (in collaboration with Antonella Di Pizio's group, Leibniz-LSB@TUM), the ligand binding pocket of LORE is located in the second lectin-like domain. Indeed, loss-of-function mutations in LORE's ligand-binding domain are consistent with the predicted binding pocket model. Recent molecular dynamics modeling has provided insights into the potential entry point and binding mode of 3-OH-FAs. This will deepen our understanding of how chain-length-specific sensing of 3-OH-FAs occurs and how ligand binding ultimately leads to the activation of LORE and downstream signaling.

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Poster N° 6 – Flash Talk (part 1)

Forces at Play; Spatio-temporal dynamics of JEDI-1, a putative mechanosensitive RLK in A. thaliana.

Tanguy Heesemans*, Cecilia Borassi, Mark Roosjen, Yosapol Harnvanichvech, Dolf Weijers, and Joris Sprakel

Plants cells are known to respond to mechanical stimuli, yet the molecular mechanisms involved in mechanical sensing are not completely understood. Here we present JEDI-1, a putative strectch activated mechanosensitive kinase. JEDI-1 has an extracellular domain (predicted to bind mannose), a transmembrane domain and an intracellular kinase domain. JEDI-1 knockouts develop shorter roots and form fewer lateral roots, two processes in which mechanical forces are involved. JEDI-1 localizes at the plasma membrane and after plasmolysis JEDI-1 seems to accumulate in Hechtian Attachment Sites (the site where the Hechtian Strands connect to the cell wall). Furthermore, Fluorescence Recovery After Photobleaching (FRAP) measurements indicate that JEDI-1 is largely immobile, possibly due to strong cell wall anchoring, which is not affected by the application of an osmotic stress. FRAP on truncated versions of the JEDI protein will be performed to assess if the JEDI dynamics are governed by the extracellular domain. In vitro experiments will be performed determine the JEDI-1 epitope. Our results will help us characterize the role of JEDI-1 in plant mechanosensing.

Poster N° 7 – Flash Talk (part 1)

CrRLK1L-RALF signaling controls powdery mildew susceptibility in Arabidopsis

Sebastian Schade*, Henriette Leicher, Ralph Hückelhoven, Martin Stegmann

CATHARANTHUS ROSEUS RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) proteins are important regulators of plant growth, development and immunity. The CrRLK1L FERONIA (FER) promotes pattern-triggered immunity (PTI) and resistance to Pseudomonas syringae pv. tomato by facilitating the formation of microbe-associated molecular pattern -induced pattern recognition receptor complexes in a RALF ligand-dependent manner. By contrast, during fungal powdery mildew infections fer knockout mutants are more resistant, suggesting that FER functions as a susceptibility factor. Yet, fer mutants do not show full resistance, raising the question whether additional related receptors may contribute to FER-mediated powdery mildew susceptibility. Next to FER, the Arabidopsis genome encodes for 16 additional CrRLK1Ls and at least 37 RALFs. Recent genetic data from our group indicate that multiple RALFs also modulate powdery mildew infection, with higher order RALF mutants displaying enhanced resistance compared to fer. Upon analysis of additional CrRLK1L mutants, we obtained evidence that other members of this receptor family can promote powdery mildew colonization. Currently, we are combining genetics and biochemistry to unravel diverse CrRLK1L-RALF modules and their interplay with FER to regulate powdery mildew infection success on Arabidopsis

Poster N° 8 – Flash Talk (part 1)

Assembly of Cysteine-Rich Receptor-Like Kinase complexes

Jente Stouthamer*, Judith Lanooij, Sergio Martin Ramirez, Jan Maika, Michelle von Arx, Dushan Zivkovic, Jani Bolla, Julien Gronnier, Rudiger Simon, Elwira Smakowska-Luzan

Cysteine-rich receptor-like Kinases (CRKs) are thought to be involved in plant defence and developmental responses, but their specific signalling pathways and regulatory molecules remain unclear. CRKs are hypothesised to sense Reactive Oxygen Species (ROS) due to their cysteine-rich extracellular domain (ECD). To study the function of CRKs, we've applied multidisciplinary approaches. Sequence alignments and predictive modelling show that CRK-ECDs are structurally similar but sequentially variable, suggesting that the functions of the ECDs might differ between family members. Modelling multimer structures indicates that some CRK-ECD pairs can form dimers, among which CRK18. CRK18 homodimerization was confirmed in vitro (MST) and in vivo (Co-IP and FRET-FLIM). We investigated CRK18 cysteine to alanine mutants, targeting single disulphide bridges, to assess if they could be important for dimerization. Most mutants showed reduced stability in vitro and mislocalized in planta, except for one mutant variant. However, this cysteine mutant only influenced CRK18 homodimerization minimally, indicating cysteine residues are not crucial for dimerization. CRK18's homodimerization ability could play a role in signal transmission, as is typical for receptor kinases. However, its specific function or possible ligand remains unknown. We will employ phosphoproteomics and immunoprecipitation-mass spectrometry to study its mechanisms of activation and function further.

Poster N° 9 – Flash Talk (part 1)

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

Tora Fougner-Okland, Jonathan Jelen, Athanasios Makris, Martina Ried , Martin Parniske, Kate Parys

Malectin-like Domain Leucine-Rich Repeat Receptor-Like Kinases (MLD-LRR-RLKs) have been implicated in processes involving microbial sensing and accommodation, but their functions in these interactions are poorly characterized. The Lotus japonicus Symbiosis Receptor-Like Kinase (SymRK) is the most studied member of this receptor family and is required for epidermal infection in Arbuscular Mycorrhiza (AM) and Root Nodule Symbiosis (RNS) [1, 2, 3, 4]. Hypothesizing that SymRK-like receptors might function in the root cortex, the primary tissue where root endosymbiont accommodation occurs, 6 SymRK Homologous Receptor Kinases (SHRKs) were identified in L. japonicus and investigated for their function in AM and RNS[5]. In a preliminary analysis of LORE1 insertion mutants, we found that the phenotypes of shrk mutants differ from that of symrk, suggesting functional divergence within the gene family. In this project, we aim to characterize the evolutionary trajectories and molecular functions of SymRK gene family members using a set of comparative approaches. Our goal is to shed light on the common and divergent features of MLD-LRR-RLKs in plant-microbe interactions that drive their sub-functionalization.

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Poster N° 10 – Flash Talk (part 1)

Investigating The Role of Plant Immune Receptors in The Restriction Of Clavibacter Diseases

Charis Ramsing, Jonathan Calzada, Danielle Stevens, Raj Kumar Verma, Maoz Aizikowitz, Doron Teper, Gitta Coaker

Clavibacter is a genus of Gram-positive, xylem-colonizing bacterial plant pathogens. Clavibacter diseases impact a wide range of plant hosts including both monocots and dicots, but each Clavibacter species is specific to its host. Clavibacter can communicate with their hosts by secreting effector proteins contributing to virulence. Of these, two effector types, serine proteases and CAZymes, are known to be important. As Clavibacter cannot secrete proteins directly into plant cells; we hypothesize effectors and pathogen-induced damage are recognized by surface-localized receptors. Here I will present our current understanding of Clavibacter effectors and plans for investigating the role of receptor proteins (RPs) and receptor kinases (RKs) in Clavibacter-tomato interactions. Clavibacter effector proteases correlate with host range within the Solanaceae and can be recognized by certain family members, including tobacco and eggplant. The ChpG effector induces SOBIR1-dependent cell death, indicating recognition is mediated by RPs. CAZymes likely aid Clavibacter in movement, facilitate release from the xylem, and may activate damage or danger RKs. To study the role of damage and danger immune perception in restricting Clavibacter colonization I will inoculate genome-edited knockouts of tomato damage receptors with pathogenic and non-pathogenic Clavibacter strains and measure bacterial growth and disease severity. Confocal microscopy will be used to visualize bacterial movement and cell wall breakdown. This study will contribute to understanding of how plants control vascular pathogens and what determines disease outcomes for Gram-positive plant pathogens.

Poster N° 11 – Flash Talk (part 1)

How flexible are the leucine-rich repeat receptor-like proteins compared to receptor-like kinases?

Nandeesh Jalahalli Rangegowda*, Marie Bolger, Björn Usadel & Remco Stam

Receptor-like proteins (RLPs) and receptor-like kinases (RLKs) are major cell surface receptors in plants, playing crucial roles in regulating immunity, stress responses, and various developmental processes. We investigating the flexible nature of leucine-rich repeat (LRR) RLPs compared to RLKs in conferring resistance to biotic and abiotic stresses.

A genome-wide and comparative analysis of RLPs and RLKs was conducted in Arabidopsis, Solanum, and Lactuca species. The analysis focused on copy number variants and physical clustering of RLPs and RLKs. Additionally, an in-depth analysis of complete RLPs (those containing a signal peptide, LRR, and transmembrane domain) was performed in wild relatives of Solanum species.

The comparative analysis revealed prominent expansion and contraction of RLPs in terms of copy number variants and physical clustering compared to RLKs. Specifically, RLPs exhibited significant variability in expansion and contraction across chromosomes in wild Solanum species. These findings suggest that the diversity of RLPs is higher than RLKs when considering both complete and incomplete (lacking either a signal peptide, transmembrane domain, or both) RLPs.

The ongoing study highlights the greater diversity and variability of RLPs compared to RLKs, suggesting a more flexible and dynamic role for RLPs in plant resistance to various stresses.

Poster N° 12 – Flash Talk (part 1)

Exploring the Evolutionary Role and Agricultural Potential of Sulfated Peptides in Plants

Amalie Scheel Tost*, Frederik Grønbæk Tidemand, Anja Thoe Fuglsang

Sulfated peptides are crucial signaling molecules involved in plant growth, development, and defense mechanisms. In Arabidopsis thaliana, four distinct types of sulfated peptides— PHYTOFSULFOKINE (PSKs), PLANT-PEPTIDE CONTAINING SULFATED TYORSINE (PSYs), ROOT MERISTEM GROWTH FACTORS (RGFs), and CASPARIAN STRIP INTEGRITY FACTORS (CIFs)—have been identified. These peptides rely on tyrosine sulfation, facilitated by tyrosine protein sulfotransferase (TPST), for their full activity. Arabidopsis possesses a single TPST gene, and its knockout results in a dwarf phenotype due to the loss of sulfated peptide function. The receptors for these peptides belong to the leucine-rich repeat receptor-like kinase family (LRR-RKs), with structures and binding sites resolved for PSK receptor 1 (PSKR1), RGF receptor 1 (RGFR1), and CIF receptor GASSHO1/SCHENGEN 3 (GSO1/SGN3).

In recent years, the growth promoting abilities and involvement in plant immunity have increased interest in leveraging sulfated peptides for agricultural applications. Despite this, the presence and significance of sulfated peptides in plants beyond model organisms have yet to be comprehensively investigated.

Our research explores the evolutionary role and importance of sulfated peptides through bioinformatics analysis. We have mapped the distribution of sulfated peptides, their receptors, and TPST across different plant species, identifying key sites essential for their activity. This study provides a comprehensive overview of the functional and evolutionary significance of sulfated peptides, offering insights into their potential applications in enhancing plant resilience and growth in agriculture.

Poster N° 13 – Flash Talk (part 2)

Glutamate as a spatio-temporal integrator between mechanosensing and microtubule behaviour

Bellandi Annalisa*, Lionnet Claire, Hamant Olivier

When responding to various stimuli plants couple cellular responses to intercellular ones, leading local stimuli to affect the characteristics of tissues and organs. Upon touch, for example, plants cells respond with increasing apoplastic glutamate levels. Therefore possibly linking release of glutamate to mechanosensing and mechanotransduction. Microtubules are key in defining tissue mechanical properties, and it is known that they can dynamically respond to changes in stress patterns. However how microtubules reorient in response to stress it is not known. Similarly, it is unknown how glutamate is released upon mechanical stimulation and what the implications of this release are in the context of mechanotransduction. We hypothesise that glutamate could affect microtubule dynamics and properties via posttranslational modification (glutamylation) of tubulin, therefore representing the missing link between mechanosensing and microtubule behaviour. To test this, we investigate levels of tubulin glutamylation in plants, mechanisms of glutamate release upon touch and effects of glutamate on microtubule dynamics.

Poster Nº 14 – Flash Talk (part 2)

Identification of new molecular components involved in Arabidopsis immune responses activated by cell-wall-derived glycans

Klara Culjak, Diego José Díaz Berlanga, Marina Martín-Dacal, Antonio Molina, Miguel Ángel Torres, **Lucía Jordá***

To face microbial pathogens and pests, plants have developed sophisticated defensive mechanisms that involve different levels of recognition. Pattern-triggered immunity (PTI) is one of the robust layers of defence and is activated when membrane-located plant pattern recognition receptors (PRRs) specifically bind non-self microbe-associated molecular patterns (MAMPs) or self damage-associated molecular patterns (DAMPs) (Molina et al., 2024). Extracellular ectodomains (ECDs) of PRRs may detect a variety of ligands, including glycans from plant cell walls or microorganisms' outer layers. A genetic screening performed to identify Arabidopsis thaliana mutants impaired in glycan perception (igp), led to the identification of a new family of receptor kinase (RK) proteins, IGP1/CORK1, IGP3, and IGP4, whose ECDs contain leucine-rich repeats and malectin domains (LRR-MAL RK). These novel PRRs are required for triggering immune responses mediated by oligosaccharides derived from cellulose (e.g., CEL3 trisaccharide) and mixed linkage β-1,3/1,4-glucans (MLGs) (Martín-Dacal et al., 2023). It has been shown that IGP1 but not IGP4 binds cellulose with a high affinity, confirming IGP1's role as a plant PRR for cellulose-derived oligosaccharides and suggesting a potential role of IGP4 as co-receptor (Martín-Dacal et al., 2023). This type of screening based on an early defence response hallmark such as the measurement of changes in cytosolic calcium levels, is a very useful tool for the identification of molecular components involved in the early stages of recognition as PRRs. Specificity and genetic complementation tests have been performed to characterize additional igp mutants identified in the laboratory (igp9, igp16, igp17, igp18, and igp19). In addition, whole genome sequencing of these igp mutants has allowed the identification of new alleles of IGP1 and IGP4, confirming the relevant role of these two receptors in Arabidopsis glycan perception. Furthermore, the requirement of additional molecular components involved in plant immune responses triggered by CEL3 and MLG43 has also been determined.

Poster N° 15 – Flash Talk (part 2)

A natural phytocytokine antagonist in tomato

Lei Wang*, Nga Pham, Yan L. Wang, Andreas Schaller, Judith Fliegmann, Matthias Erb, Thomas Boller, Georg Felix

Cytokines are pivotal immunomodulators in animals and plants. Systemin, the first plant cytokine identified, mediates responses to injury and herbivory. Here, we show that tomato contains a small protein with a systemin-like C-terminus that acts as a potent natural antagonist of systemin (antiSYS). The antiSYS gene is part of a cluster with four additional genes encoding similar proteins. However, unlike antiSYS, the C-termini of three of these proteins are agonists like the original systemin, whose activities are also inhibited by antiSYS. Tomato mutants lacking antiSYS exhibit pleiotropic phenotypes, including aberrant growth and reduced production of fruits and seeds. Thus, reminiscent of antagonistic interleukins controlling inflammatory responses in animals, antiSYS in tomato is crucial for development by balancing an array of agonistic immunostimulatory systemins.

Poster N° 16 – Flash Talk (part 2)

CLE peptide signalling dynamics in the Arabidopsis shoot apical meristem during floral transition

Svenja Augustin *, Meik Thiele, Rüdiger Simon

In flowering plants, the optimal timing of floral transition is crucial for reproductive success. During this developmental phase change, the shoot apical meristem (SAM) ceases to produce leaf primordia and starts to initiate floral organ primordia instead. Previous research in Arabidopsis thaliana has shown that floral transition is associated with changes in SAM morphology. While the vegetative meristem is small and flat, the onset of reproductive growth induces a transient increase in both meristem volume and curvature. This results in an inflorescence meristem (IFM) that is both larger and more curved than the vegetative SAM. Apart from the developmental stage, different CLE (CLAVATA3/EMBRYO SURROUNDING REGION-RELATED) peptides have been identified as key regulators of SAM size and shape. In the IFM, CLV3 and CLE40, which are expressed in the stem cell zone and the meristem periphery respectively, regulate the expression of the stem cell-promoting transcription factor WUSCHEL (WUS) and thereby influence meristem shape. However, how the dynamic morphology of the Arabidopsis shoot apical meristem during floral transition relates to potential changes in CLE signalling remained unknown.

Using confocal microscopy, we found that CLE40 promotor activity is drastically altered during floral transition. A quantitative, 3D analysis of CLV3 and WUS expression in Col-0 and cle40 demonstrated the importance of CLE signalling during this process and, additionally, revealed that cle40 mutants initiate floral organ primordia earlier. These observations were further substantiated in phenotyping experiments and suggest a novel function of CLE40 in floral transition, thus providing a link between stem cell homeostasis, CLE signalling and the developmental stage of the plant.

Poster N° 17 – Flash Talk (part 2)

Cell surface receptors and quantitative resistance in lettuce

Iñigo Bañales*, Sarah Mehrem, Marrit Alderkamp, Samara Almeida Landman, Alexander Kozik, Bart Schimmel, Richard Michelmore, Basten Snoek, Dmitry Lapin, Guido van den Ackerveken.

Cell surface receptors sensing molecules derived from or associated with microbial pathogen invasion (PAMPs) allow plants to mount an effective immune response (PAMP-triggered immunity, PTI). Outputs of PAMP recognition include reactive oxygen species (ROS) burst, mitogen-activated protein (MAP) kinase phosphorylation, and host transcriptional reprogramming. Surface immune receptors can confer broad-spectrum resistance, since PAMPs are often shared between distinct pathogens. PTI has been proposed as the basis for quantitative disease resistance (QDR), defined as an incomplete, durable resistance mediated by multiple loci. Importantly, it is becoming increasingly evident that PTI is also required for qualitative (or full) resistance conferred by intracellular immune receptors. Altogether, PAMP receptors represent a promising lead for resistance engineering in crops.
Despite being an attractive lead for breeding, there is limited knowledge on how PTI works and connects to other sectors of immunity in species other than Arabidopsis thaliana. Lettuce is an ideal candidate to fill this gap. Research on lettuce has advanced significantly in the recent years with the establishment of efficient transformation protocols and high-quality genome resources. There is a strong interest to unravel PTI mechanisms in lettuce due to the increasing emergence of resistance-breaking pathogen isolates. One of the most severe diseases is caused by the oomycete Bremia lactucae. Bremia can quickly circumvent the qualitative effect of newly introgressed Resistance (R) genes, many of which map to loci encoding intracellular immune receptors. In this context, the use of PTI emerges as a promising approach to boost lettuce immunity. However, PAMP receptors in lettuce are virtually unknown and PTI in lettuce is not well understood.

Through this research, we aim to learn about conserved and distinctive features of PAMP-triggered immunity in lettuce, which is a model species for the large Asteraceae plant family and to inform breeding efforts for quantitative disease resistance. To this end, we combine a variety of techniques, including association mapping, comparative genomics, genome editing, genetic complementation assays and transcriptomics.

Poster N° 18 – Flash Talk (part 2)

Control of the transcript levels of the immune receptors via salicylic acid in unchallenged plants

Tijmen van Butselaar, Savani Silva, **Dmitry Lapin**, Iñigo Bañales, Sebastian Tonn, Chris van Schie, and Guido Van den Ackerveken

Immune receptors of plants detect pathogen-derived molecules and activate immune responses. The work of multiple laboratories helped to resolve details of the signaling events from receptor activation to Ca2+ influx and transcriptional mobilization of defense. Basal receptor expression levels (in unchallenged plants) are important for effective immunity activation. However, details on how this is regulated still need to be clarified. In the study of the salicylic acid (SA) inactivating hydroxylases DMR6 and DLO1 from Arabidopsis, we obtained Arabidopsis plants with low basal SA levels. These lines displayed low levels of transcripts of ~30% immune receptors, both plasma membrane and intracellular ones. In particular, the low SA levels in Arabidopsis plants were associated with the lower level of the expression and signaling outputs of the RECEPTOR-LIKE PROTEIN 23 (RLP23), which is a receptor for a pathogen-associated molecular pattern (PAMP) nlp24 found in several microbial kingdoms. The RLP23 expression and signaling were similarly dependent on the SA receptors NPR1/NPR4. Next to these findings, we will present initial unpublished data from the ongoing work connecting SA perception to the RLP23 transcription control, where we test transcription factors as candidate regulators of the RLP23 signaling (reactive oxygen species, ROS, burst). Overall, our data identify the defense hormone SA as a critical factor regulating the expression of ~30% of immune receptor genes in unchallenged Arabidopsis plants.

Poster N° 19 – Flash Talk (part 2)

An Endogenous Peptide PEP2 modulates Iron-Deficiency Signaling and Root Growth in Arabidopsis.

Deep Shikha and **Santosh Satbhai***

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. Here we report that danger associated molecular pattern such as AtPROPEP2 is significantly induced under Fe deficiency which lead to modulation of expression of Fe-related genes. PEP2 is perceived by PEPR2 to positively regulate ROS content and negatively regulate primary root growth, iron content and rhizosphere acidification. Collectively, these data indicate that AtPep2 plays a crucial role in Fe deficiency signalling pathway in plants.

Poster N° 20 – Flash Talk (part 2)

Mineral nutrients affect RALF-pectin condensation and subsequent RALF signaling processes in plant roots

Paulina Ramirez Miranda*, Rößling Ann-Kathrin, Simon Stitzinger, Ibrahim Cissé, Jürgen Kleine-Vehn, Elke Barbez

In plants, receptor-ligand interactions play pivotal roles in mediating responses to environmental stimuli. Our research focuses on understanding how mineral nutrient homeostasis in the root apoplast impacts cellular signalling pathways. Specifically, we study the interactions involving RAPID ALKALINIZATION FACTORs (RALFs) and the FERONIA receptor.

Recent studies indicate that the degree of pectin methyl esterification is critical for orchestrating these signalling events through RALF-pectin condensate formation (Rössling et al., 2024 Elife; Liu et al., 2024). On the other hand, our unpublished work reveals that cationic mineral nutrients interact with negatively charged pectin in the cell wall. Therefore, we investigate how the environmental availability of mineral nutrients influence RALF-pectin condensate formation and the subsequent RALF/FERONIA signaling outputs.

Our findings reveal the intricate interplay between mineral nutrient status in the apoplast and the sensitivity of plants to RALF-mediated signalling. Our work sheds light on novel mechanisms underlying plant responses to environmental cues. By elucidating these mechanisms, our research contributes to a deeper understanding of receptor-ligand interactions in plants and their significance in adaptive responses to changing environmental conditions.

Poster N° 21 – Flash Talk (part 2)

Identification of cell wall regulators in plant root regeneration

Yunjing Ma*, Lieven De Veylder

Regeneration is a remarkable biological process through which damaged tissues or organs can restore their complete structure and functionality, serving as a self-preservation mechanism. In our previous research, we identified an Arabidopsis thaliana transcription factor, ETHYLENE RESPONSE FACTOR115 (ERF115) that plays a crucial role in orchestrating the replenishment of lost cells or tissues following injuries (Heyman et al., 2013; Heyman et al., 2016). Through a pharmacological screen, we identified Epigallocatechin-3-Gallate (EGCG) as a potent inducer of promoter activity of both ERF115 and its closest homolog ERF114. EGCG blocks esterase activity of pure pectin methyl esterase (PME) as well as PME extracts from citrus in vitro (Lewis et al., 2008). Therefore, it is plausible that the changes induced by EGCG in pectin biochemistry are closely linked to the transcriptional responses of both ERF114 and ERF115, ultimately influencing the regeneration process. Notably, our research has unveiled that EGCG-induced expression of ERF114 and ERF115 correlates with a 76% increase in lateral root (LR) density without inhibiting primary root growth, strongly suggesting that the de novo formation of roots is a consequence of alterations in pectin biochemistry. While the specific PME(s) among the 69 PMEs that are inhibited by EGCG remain unidentified, and the exact nature of the other pathways affected by EGCG is yet to be determined; we hence performed an RNAseq experiment. Differential gene expression analysis identified 4755 upregulated genes at 6h, with 3389 genes overlapping with those upregulated at 12h. Additionally, 1366 genes were exclusively upregulated at 6h, representing early responsive genes, while 1036 genes were uniquely upregulated at 12h, indicative of late responsive genes. GO enrichment analysis highlighted that genes upregulated at 6h were associated with pathways such as oxidative stress and defense response to bacteria, whereas the top 20 clusters of downregulated genes were related to cell cycle processes and noncoding RNA functions.

Poster N° 22 – Flash Talk (part 2)

S^2-PepAnalyst: A Web Tool for Predicting Plant Small Signalling Peptides

Kelly L. Vomo-Donfack, Mariem Abaach, Ana M. Luna, Grégory Ginot, Verónica G. Doblas, **Ian Morilla***

Small Secreted Peptides (SSPs), essential to plant biology, are known to regulate a large diversity of biological processes, including growth, development, stress responses, and pathogen interactions [1]. Despite their importance, predictive tools for SSPs are lacking. S2-PepAnalyst fills this gap (http://www.s2-pepanalyst.uma.es), offering a comprehensive platform for predicting SSPs and identifying similarities within peptide families. Users can input sequences (2-200 AAs) for analysis. Our model transforms input sequences into images using Large Language Models (LLM), leveraging cleavage sites from SignalP6.0 pre-training, and innovative GeoTop image-based topological transformer techniques for precise predictions [2-4]. This tool, tested on datasets from avocado, Arabidopsis thaliana, mango, and tomato, integrates Convolutional Neural Networks (CNN), and local reinforcement learning for robust, accurate predictions, enhancing our understanding of peptide functions. S2-PepAnalyst will facilitate the discovery of novel SSP families in non-model plant species.

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Poster N° 23– Flash Talk (part 2)

CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASE2 – gateway to the intercellular communication

ZEINER Adam*, WRZACZEK Michael

Receptor-like protein kinases (RLKs) are the key component involved in the sensing of the extracellular micro-environment. One of the biggest group of RLKs, cysteine-rich RLKs (CRKs), is involved in the response to developmental and stress-related factors, although the molecular mechanisms of their functions are not yet elucidated. The composition of the plasma membrane (PM) is not uniform, and lateral distribution of its components predestines the existence of PM domains. The presence of characteristic lipids and proteins, including members of RLKs, plays a role in the determination of the function of those PM domains. Plasmodesmata (PD), one type of PM domain, are channels connecting almost all plant cells. PD allow the intercellular transport of various molecules, thus impacting biological processes. Our research is focused on CRK2 – one of the best described member of CRK family. CRK2 oppositely regulates the levels of PD-localized callose deposits in the response to flg22 and salt stress. Moreover, CRK2 positively regulates the RBOHD-dependent production of reactive oxygen species (ROS), potent modulator of callose deposition. Importantly, CRK2 is enriched at PD upon flg22 and salt stress, interacts with the predominantly PD-localized CALLOSE SYNTHASE1 (CALS1) in vivo, and interacts and phosphorylates CALS1 in vitro. Our goal is to describe the impact of CRK2-mediated phosphorylation of CALS1 and subsequent physiological effect on response to the presence of flg22 and salt.

Poster N° 24

Identification of signalling components regulating floral organ abscission

Sergio Galindo-Trigo, Virendrasinh Khandare, Mark Roosjen, Julian Adams, Alexa-Maria Wangler, Martin Bayer, Jan Willem Borst, Veslemøy Kaen, Elwira Smakowska-Luzan, **Melinka A. Butenko***

Cell separation during the shedding or organs is a tightly controlled developmental programme, such as what occurs during abscission of floral organs post pollination. 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 receptor-like 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 characterize brassinosteroid signalling kinases (BSKs) as regulators of floral organ abscission. BSK1 localizes to the plasma membrane of abscission zone cells and interacts with HAESA receptors. We show that the identified abscission-promoting allele of BSK1 also enhances receptor signalling in other BSK-mediated pathways, suggesting conservation of signalling mechanisms. Furthermore, preliminary results from a forward genetic screen conducted on the ida-1 mutant background identify a potential new transcription factor acting downstream of the HAESA signalling system.

Poster N° 25
Functional recharacterization of BIR2 in pattern-triggered immunity

Kyle W. Bender, Achchuthan Perinpanathan, Henning Mühlenbeck, Laura Herold, Emma Six, Cyril Zipfel

In plants, plasma membrane-localized receptor kinases (RKs) and receptor-like proteins serve as the primary surveyors of the extracellular space for molecules of both self and non-self origin that regulate internal developmental programs and responses to environmental stimuli. Receptor complex activation and consequently downstream signaling is controlled by a suite of accessory RKs but the biochemical mechanisms governing accessory RK function remain largely undefined. In a quantitative proteomics analysis, we observed the ligand-triggered association of the accessory RK BAK1-INTERACTING RECEPTOR KINASE 2 (BIR2) with the pattern-recognition receptor (PRR) ELONGATION FACTOR Tu RECEPTOR (EFR). This seemingly contradictory observation prompted us to revisit the proposed function of BIR2 as a negative regulator of PRR complexes. Using three novel deletion mutants generated by CRISPR/Cas9-mediated gene editing, we demonstrate that the loss of BIR2 alters elf18 but not flg22 or SCOOP12 responses in vegetative tissues. To gain further insight into BIR2 function, we evaluated BAK1 complexes containing BIR proteins by quantitative proteomics and found that BIR2 and BIR3 are present in BAK1 affinity enrichments at similar stoichiometries while BIR1 and BIR4 are represented at only low abundance, hinting at a possible tripartite complex consisting of BAK1, BIR2, and BIR3. Consistently, BIR2 interacts with BIR3 in split luciferase assays. Collectively, our work suggests a specific role for BIR2 in the regulation of EFR-mediated elf18 responses. Current work aims to understand, mechanistically, the role of BIR2 during elf18-triggered immune responses and to unravel the role of a putative BAK1-BIR3-BIR2 complex in immune system regulation.

Poster N° 26

Unravelling the mechanisms of Nod factor-independent root-nodule symbiosis in Aeschynomene evenia: the role of AeCRK and AeRLCK2.

David Landry*, Natasha Horta Araújo, Léandre Bouat, Carole Pichereaux, Johan Quilbé, Benoit Lefebvre, Jean-François Arrighi

Most plants establish a mutualistic interaction with arbuscular mycorrhizal (AM) fungi, while legumes also engage in symbiosis with nitrogen-fixing rhizobia to form the root-nodule (RN) symbiosis. The establishment of the RN symbiosis is well described in model legumes and typically relies on mutual recognition through a specific molecular dialogue1. Plant plasma membrane receptors of the LysM-RLK family are crucial for the perception of bacterial signals called Nod factors (NFs)2, leading to the formation of a specialised organ on the root: the nodule1. Recently, Aeschynomene evenia has emerged as a model for the study of an original NF-independent RN symbiosis that is triggered by Bradyrhizobium strains lacking the genes required for NF synthesis2,3. A forward genetic screen following EMS mutagenesis has identified novel plant players involved in this NF-independent symbiosis. No genes encoding for LysM-RLKs were identified, but two proteins, AeCRK and AeRLCK2, corresponding to a cysteine-rich receptor kinase and a receptor-like cytoplasmic kinase, respectively, were discovered as early symbiotic players4,5. Interestingly, AeCRK has no direct equivalent in model legumes and AeRLCK2 is an Aeschynomene-specific duplicated gene whose closest homologs in Lotus japonicus are LjAMK8 and LjAMK24, two key AM players4,6. However, both AeCRK and AeRLCK2 are dispensable for this symbiosis. AeCRK and AeRLCK2 are proposed to have evolved to mediate the NF-independent RN symbiosis4,5.
The AeCRK and AeRLCK2 protein families are key players in plant signaling pathways and are often involved in receptor complexes. To test the possibility that AeCRK and AeRLCK2 form such a receptor complex, we first assessed the predictions that these proteins are both located at the plasma membrane and have active kinase domains. Then, by combining different biochemical approaches, we showed that they physically interact together. AeCRK has the strongest kinase activity and transphosphorylates AeRLCK24. To decipher the AeRLCK2/AeCRK proximal proteomes in the presence/absence of Bradyrhizobium, a proximity-labelling approach (TurboID-based experiments) was also performed. Potential partners were successfully identified and are currently being investigated for their symbiotic involvement through functional studies. In complement, phosphoproteomic approaches are being set up in the presence/absence of Bradyrhizobium to identify proteins targeted by the strong kinase activity of AeCRK. Altogether, the obtained and forthcoming data will help us to progressively unravel the signaling pathway that mediates the NF-independent RN symbiosis.

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Poster N° 27

Cysteine-rich Receptor-like Kinase 7 functions as a receptor for Wall Teichoic Acid, a MAMP specific for gram-positive bacteria with non-canonical activities in Arabidopsis thaliana.

Leon Pierdzig*, Christine Trippel, Samantha Armiento, Lisa Schulz, Jeanine Rismondo, Cristina De Castro, Antonio Molinaro, Elena Petutschnig, Volker Lipka

Plants utilize a variety of cell-surface receptors, sensing alterations in the microbiota composition via perception of intercellular Microbe Associated Molecular Patterns (MAMPs). Perception of bacterial MAMPs has been well studied utilizing the gram-negative model organisms Pseudomonas syringae. However, our knowledge about gram-positive bacterial MAMPs remains limited. Teichoic acids are large, anionic glycopolymers specific for the cell wall of gram-positive bacteria and are required for bacterial cell elongation, cell division, and overall fitness. Here, we demonstrate that the perception of wall teichoic acids (WTA) from different gram-positive bacteria can elicit non-canonical defense responses in the form of programmed cell death in Arabidopsis. We provide genetic evidence that plant perception of WTA and subsequent induction of defense responses is dependent on a Cysteine-rich Receptor-like Kinase 7 (CRK7). CRK7-dependent recognition of WTAs results in the expression of Salicylic Acid (SA) marker genes in local and systemic tissues. Further genetic analyses indicate that sugar modification of the WTA backbone is required for recognition and subsequent induction of defense responses. In conclusion, Arabidopsis perceives glycosylated WTAs from gram-positive bacteria and induces non-canonical defense responses.

Poster Nº 28

The role of the receptor like cytoplasmic kinase (RLCK) class VII, subfamily 7 in immunity of Nicotiana benthamiana

Huan Liu, Wen R.H. Huang, Matthieu H.A.J. Joosten

Tomato (Solanum lycopersicum) leaf mould is a fungal disease caused by Fulvia fulva (Cladosporium fulvum). In response to this pathogen, tomatoes have evolved several receptor-like proteins (RLPs) that recognize specific effectors secreted by F. fulva, making these effectors avirulence factors (Avrs). One well-characterized RLP is Cf-4, which detects the Avr4 effector from F. fulva. It is well established that receptor-like cytoplasmic kinases (RLCKs) play crucial roles in immunity in Arabidopsis thaliana. However, little is known about the signaling proteins downstream of Cf-4 in Nicotiana benthamiana and tomato. In A. thaliana, RLCK class VII, which is divided into nine subfamilies, is involved in immunity. Our research result has shown that members of RLCK-VII-7 are required not only for Avr4/Cf-4-triggered reactive oxygen species (ROS) production but also for the hypersensitive response (HR) in N. benthamiana. Additionally, N. benthamiana RLCK-VII-7 members are essential for host resistance to Phytophthora palmivora. However, the downstream components of these RLCKs and their functional mechanisms remain to be studied.

Poster Nº 29

The role of StCLE17 peptide in potato tuber development

Yun Zheng, Xiaoxu Li, Sara Bergonzi, Marian Oortwijn, Richard G.F. Visser, Yongfeng Guo

CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) peptides play central roles in regulating plant development, stem cell homeostasis in different plant meristems, and cell division/differentiation through binding to leucine-rich repeat receptor-like kinases (LRR-RLKs). The diverse roles of CLE members in plant growth and development make them potential targets for crop improvement.

Potato (Solanum tuberosum L.) is a vital food commodity worldwide; however, its yield is constantly threatened by various stresses.In a previous study, several members of the potato CLE gene family (StCLE) were characterized, with comprehensive analysis of their phylogenetic relationship, structures, and expression patterns. However, detailed information about the function of the CLE family genes in potato tuber development is lacking.

In our research, we investigated the StCLE17 gene, which may be involved in regulating potato tuber development. We found that the StCLE17 gene is specifically expressed in the tuber and stolon, as determined by qRT-PCR and GUS staining, suggesting a role for its encoded protein in tuber development. We assessed StCLE17-OE (overexpression) and StCLE17-RNAi (RNA interference) lines and found that, compared to wild-type plants, StCLE17-OE lines exhibited faster stolon growth within the same time period and resulted in increased tuber mass. In contrast, StCLE17-RNAi lines exhibited the opposite phenotype. Additionally, an increased tuber size was observed when synthetic StCLE17 peptide was exogenously applied to four different genotypes of potato plants, similar to the effects of StCLE17 overexpression. These results indicate that the StCLE17 peptide influences potato stolon development and tuber weight. In future experiments, we aim to further investigate the role of the peptide and to identify its receptor protein from the StLRR-RLK gene family.

Poster N° 30

Elucidating the sensing and response to nickel of the Catharanthus roseus RECEPTOR LIKE KINASES 1 LIKE (CrRLK1L) family member THESEUS1

Fariha Naz Apon*, Julia Richter, Marie-Theres Hauser

Plant cell wall can be viewed as a platform of perceiving stress where cell wall associated receptors such as Catharanthus roseus RECEPTOR LIKE KINASES 1 LIKE (CrRLK1L) family members can sense and process stress signals. Due to anthropologic activities nickel (Ni2+) is increasing above its typical range between 10-40 µg/g in agricultural soil. Our ionome analysis detected very low levels of Ni2+ in Arabidopsis seedlings cultivated on Hoaglands medium. We and others have recently shown that increased Ni2+ ions inhibit cell elongation in hypocotyl and root growth in Arabidopsis thaliana (Richter et al., 2017). We also demonstrated that the CrRLK1L member, THESEUS1 (THE1), is involved in growth responses to excess Ni2+ concentrations starting at 5 µM.

One of the ligands of CrRLK1Ls are the RAPID ALKALINIZATION FACTORs (RALFs) (Abarca et al., 2021). Gonneau et al., (2018) showed that THE1 is the receptor for RALF34 and the extracellular domain is binding RALF34. In order to determine if RALFs are involved in Ni2+ dependent responses we measured hypocotyl elongation of RALF34 mutants and root growth of the nonsense mutant the1-6 with other RALFs. The results will be discussed in respect to the upstream sensing events of the Ni2+-THE1 signaling cascade.

To understand the earliest response to increased Ni2+ concentrations we quantified the activity of root meristems using the mitotic cell division reporter line CycB1,1::CDB-GUS and observed dynamic changes which finally results after 6 hrs in a reduced mitotic activity upon Ni2+. Based on these time course experiments we performed phospho-proteomic analyses comparing wildtype and the1-6 with and without Ni2+. We will present the phosphoproteome analysis after 5 min and 30 min of exposure to Ni2+ and discuss the data in respect to downstream signaling events.

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Poster N° 31

Investigating peptide hormones in fruit ripening through a CRISPR/Cas9-mediated multiple knockout strategy

Marco Boschin*, Carlotta Francese, Alessandra Bellan, Anna Pavanello, Marco Armellin, Livio Trainotti

Food spoilage and waste are critical problems nowadays, with post-harvest losses having a profound impact on fruit and vegetable production. Addressing this huge issue requires a deeper and more comprehensive understanding of the mechanisms that drive ripening, as well as the intricate networks of biological signals that regulate these processes.

Our project aims to understand the still poorly understood role of plant peptide hormones (PHs) in controlling the development and ripening of fleshy fruits. Indeed, PHs are fundamental signaling biomolecules in plant cells, already known for their ability to regulate various developmental and defense processes.

To study PHs functions, we started by deepening the understanding of different PHs signaling in various crops, looking at the expression fluctuations of PHs in multiple tissues of plants. Then, we adopted a genome editing strategy that allows us to generate Solanum lycopersicum lines characterized by CRISPR/Cas9-mediated multiple knock-outs of PHs genes of the CLAVATA3/EMBRYO-SURROUNDING REGION (CLE) family. This approach will enable us to perturb specific nodes in the biological signaling network that regulate the growth, development, and ripening of fleshy fruits and will allow us to understand the combinations of peptides involved in these processes. The results will be descriptive of the phenomenon, allowing us to avoid the emergence of complementation effects and providing a description of the contribution of each peptide during ripening.

Poster N° 32

Dual function of damage peptide receptor PEPR: promoting both defense and growth during adaptation to phosphate deficiency

Natsuki Tsuchida*, Kota Yamashita, Taishi Umezawa, Yusuke Saijo

Under phosphate (Pi) limitation, plants engage in mutualistic microbes for increasing Pi acquisition, yet mount pathogen resistance via the mechanisms poorly understood. In Arabidopsis thaliana, recognition of damage-inducible Pep peptides by the leucine rich repeat receptor kinases (RKs) PEPR1/PEPR2 leads to activation of defense signaling toward pathogen resistance. We show that defense-related transcriptional responses to Pep1 through PEPRs, but not to bacterial flagellin or fungal chitin, is sensitized in Pi-deprived seedlings. This points to functional diversification of pattern recognition receptors under Pi deficiency. Notably, Pi starvation response (PSR)-related transcriptional reprogramming under low Pi is reduced in non-elicited pepr1 pepr2 plants. Our results suggest that the PEPR pathway not only strengthens defense responses but also contributes to plant growth and may thus be favored during adaptation to phosphate deficiency.

Poster N° 33

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

Vahid Fallahzadeh-Mamaghani, Sarina Schulze, Liping Yu, Chenlei Hua, Lisha Zhang, Dagmar Kolb, Hannah Weber, Alexandra Ehinger, Svenja C. Saile, Mark Stahl, Mirita Franz-Wachtel, Lei Li, Farid El Kasmi, Thorsten Nürnberger, Volkan Cevik, Birgit Kemmerling

Arabidopsis BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) is a key player in mediating pattern-triggered immunity (PTI) as a co-receptor of leucine-rich repeat (LRR) pattern recognition receptors (PRRs). Genetic manipulation of BAK1 and BAK1-INTERACTING RECEPTOR KINASES (BIRs) results in uncontrolled cell death. BIR3 interacts physically with the TIR-NBS-LRR protein (TNL) CONSTITUTIVE SHADE AVOIDANCE 1 (CSA1) and in the absence of BAK1 and BIR3, CSA1 is de-repressed and induces ETI-type cell death leading to autoimmunity. The bacterial effector HopB1 cleaves activated BAK1, triggering cell death in a CSA1-dependent manner. Cell death reactions induced by the microbial pattern pg23 rely on CSA1, but typical PTI responses are unaffected in csa1 mutants indicating that in addition to PTI responses a TNL-mediated ETI-type cell death pathway is activated by microbe-associated molecular patterns (MAMPs). Flg22 or pg23 both enhance BAK1 cleavage, suggesting that MAMP perception activates BAK1 processing. Novel insights on how CSA1 is kept under control by BIR3/BAK1 and how it is activated in their absence will be presented.

Poster N° 34

Unveiling the molecular interplay between phospho-sensors and calmodulins in regulating early LORE immune signaling in plants

Aimen Sultan* and Stefanie Ranf

The invasion of plants by microorganisms initiates a series of defense-related cellular responses mediated by pattern-recognition receptors (PRRs). The initial perception of microbe-associated molecular patterns (MAMPs) leads to the phosphorylation and activation of the cytosolic kinase domain of receptor-like kinase (RLK)-type PRRs, initiating various signal transduction pathways propagating through phospho-relay mechanisms. In Arabidopsis thaliana, the S-domain (SD)-type RLK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) serves as a PRR that activates plant immunity by binding and responding to microbe-associated 3-hydroxy fatty acids. Although LORE plays a critical role in triggering plant defensive mechanisms, e.g. by upregulating the production of reactive oxygen species (ROS) and calcium signalling, the detailed mechanisms underlying its phosphorylation-mediated downstream signaling and regulation remain largely unknown. The aim of our study is to unveil the regulatory mechanism of LORE phosphorylation, and to discover whether the molecular interplay between calcium signalling and phosphosensors regulate early LORE immune signaling. To this end, we study predicted binding site motifs for calcium (CaM) and phospho-sensors (GRFs) in the cytosolic domain of LORE and assess interaction partners of LORE through protein-protein interaction and genetic analyses. This will deepen our understanding of LORE-activated plant immunity.

Poster N° 35

Antagonistic RALF peptides control an intergeneric hybridization barrier on Brassicaceae stigmas

Zijun Lan*, Zihan Song, Zhijuan Wang, Ling Li, Yiqun Liu, Shuaihua Zhi, Ruihan Wang, Jizong Wang, Qiyun Li, Andrea Bleckmann, Li Zhang, Thomas Dresselhaus, Juan Dong, Hongya Gu, Sheng Zhong, Li-Jia Qu

Pollen-pistil interactions provide interspecific/intergeneric prezygotic hybridization barriers in plants. Rejection of undesired pollen at the stigma is critical to prevent outcrossing but can be overcome with support of mentor pollen. The mechanisms underlying this hybridization barrier are largely unknown. Here in Arabidopsis, we show that FERONIA/CURVY1/ANJEA/HERK1 receptor-like kinases and LRX3/4/5 cell wall proteins interact at the surface of papilla cells 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), as a key, outcompete sRALFs allowing pollen tubes to penetrate. Treatment of Arabidopsis stigmas with synthetic pRALFs unlocks the barrier and allows penetration of pollen tubes from distantly-related Brassicaceae species leading to the formation of interspecific/intergeneric hybrid embryos. Therefore, we uncover a 'lock-and-key' system that controls hybridization breadth of interspecific/intergeneric crosses in Brassicaceae. Manipulation of this system has the potential to facilitate wide hybridization in crops.

Poster N° 36

Production of modified plant signaling peptides with heterologous TPSTs

Kasper Di Renzo*, Frederik Grønbæk Tidemand, Anja Thoe Fuglsang

Since the discovery of systemin as the first plant signaling peptide in the early 1990's, more than 1,000 putative peptides have been reported in Arabidopsis alone. Those characterized are often post-translationally modified, including tyrosine sulfations, proline hydroxylations, and glycosylations. Tyrosine sulfation, in particular, is crucial for the bioactivity of specific plant peptide families that promote root cell proliferation and elongation. Previous studies have incorporated sulfated tyrosines into the translational machinery using expensive, chemically synthesized non-canonical amino acids. Instead, we focus on tyrosylprotein sulfotransferases (TPSTs), the enzymes responsible for post-translational tyrosine modification in plants. However, TPSTs are poorly conserved across species, and their substrate specificity remains unclear due to ambiguous recognition motifs for tyrosine modification.

Using Escherichia coli as expression host, we have been unable to produce the TPST of A. thaliana. Instead, we successfully expressed and purified two insect TPSTs in E. coli. Initial in vitro activity assays indicate that these TPSTs de indeed exhibit sulfotransferase activity against selected unmodified peptides known to be sulfated in planta.

Currently, we are co-expressing these TPST candidates with non-mature peptides to determine if in vivo sulfation can occur. This could pave the way for the development of biofactories capable of producing high-value, biologically active peptides that are otherwise economically unfeasible to synthesize.

Poster N° 37

Identification of a novel interactor family of CLE receptors

Salves Cornelis*, Kyle Bender, Samy Carbonnel, Morgane Gull, Cyril Zipfel, Ora Hazak

Receptor-like kinases BARELY ANY MERISTEM1-3 (BAM1-3) perceive peptide ligands of the CLV3/EMBRYO SURROUNDING REGION-related (CLE) family. Recognition of peptides involves the formation of receptor complexes, which are comprised of (co)-receptors and/or intracellular partners. These complexes regulate downstream processes to control development. One of such processes is xylem differentiation. No specific partners of CLE receptors had been identified to play a role in xylem maturation.

Here, we used a LC-MS/MS analysis to identify a novel RECEPTOR-LIKE CYTOPLASMIC KINASE to be interacting with BAM1, which was termed BAM1 INTERACTING RECEPTOR KINASE1 (BIRK1). Further analysis showed a specific conserved interaction between BIRK1 and other bona fide CLE receptors. Phylogenetic analysis revealed this protein was established in early angiosperms and 5 additional homologs were identified in Arabidopsis that also show interaction to BAM1. Among BIRKs, BIRK1 is most abundantly expressed and shows a wide expression domain in Arabidopsis roots. Still, overlapping expression patterns in some tissues within the BIRK family suggests possible functional redundancy. Mutant analysis of BIRK1 loss-of-function shows xylem phenotypes resembling those of CLE-signalling mutants, suggesting its key role in mediating CLE signals during xylem maturation. Conservation of BIRKs across species was also assessed.

I will present the latest results regarding this novel family of BAM interactors.

Poster Nº 38

The role of cysteine oxidative modifications of Cysteine Rich Receptor Kinases

Virendrasinh Khandare*, Sjef Boeren, Guadalupe Espadas, Eduard Sabido, Elwira Smakowska-Luzan

Plants produce Reactive Oxygen Species (ROS) in the apoplast as a universal stress response, crucial for intercellular signaling and stress adaptation. However, the specific apoplastic ROS sensors in plants and the mechanisms by which ROS perception influences protein-protein interactions and downstream signaling pathways remain unclear. This study focuses on the family of Cysteine Rich Receptor Kinases (CRKs) as promising candidates for apoplastic ROS sensors. CRKs are characterized by two Domains of Unknown Function 26 (DUF26) in their extracellular domain (ECD), featuring a conserved cysteine motif (C-8x-C-2x-C). Using redox-mass spectrometry approaches, we demonstrated that the cysteines in the ECDs of CRKs undergo oxidative modifications. The occurrence of these modifications modulates protein-protein interactions and subsequent cellular responses.

Poster N° 39

Finding of the potential role of PSY peptide family in Arabidopsis development

Jinhua Lin*, Amalie Scheel Tost

Recently the secreted peptides have been reported to act as an important role in cell to cell communication and plant development. Arabidopsis PLANT PEPTIDE CONTAINING SULFATED TYROSINE (PSY) family consists of 9 members, which are characterized by a conserved PSY domain at the C terminus. In the PSY peptide family, only PSY1 has been partly studied which is an 18-amino-acid glycopeptide, and involved in the regulation of root growth via increasing cell expansion and elongation. However, the function of eight additional homologs of PSY1 in Arabidopsis are remain to be elucidated. In this work, we try to explore the potential function of PSY peptides by three parts. Firstly, the expression pattern of PSY peptide family were investigated by genevestigator and qRT-PCR in our lab previous study. According to the results, the PSY peptides were divided into 3 groups, group I, the peptide mainly expressed in root; group II, the peptides primarily expressed in the aerial part of Arabidopsis; group III, the peptides expressed nearly equal in the green part and root. The PSY reporter lines were constructed as well to further investigate the tissue expression of PSY peptide family. Secondly, creation and identification of the phenotype of the single and high order PSY peptide mutants. The double or triple PSY mutants were created based on the expression pattern analyzed by genevestigator and qRT-PCR. Finally, verification the phenotype of each of the PSY overexpression line. Here, the phenotype verification mainly focus on Arabidopsis root development.

Poster N° 40

Exploring the Regulatory Network of Cysteine-Rich Receptor Kinases in Arabidopsis

Judith Lanooij*, Jente Stouthamer, Colin Ruprecht, Hanne Zilmer, Dirk Walther, Fabian Pfrengle, Elwira Smakowska-Luzan

Cysteine-Rich Receptor Kinases (CRKs) form a large receptor kinase group in Arabidopsis. CRKs are characterized by the presence of two cysteine-rich Domains of Unknown Function 26 (DUF26) in their extracellular domain with a conserved cysteine motif, C-8X-C-2X-C, and additional non-conserved cysteine residues. CRK receptors are limitedly studied, but it has been suggested that CRKs are involved in perceiving reactive oxygen species, through oxidative modifications on cysteine residues. This study investigates potential regulators of CRK receptors, specifically focusing on glycans and Cysteine-Rich Repeat Secretory Proteins (CRRSPs). Mannose, a glycan, is known to be bound by ginkbilobin-2, an antifungal protein from Ginkgo biloba that contains one DUF26. This, along with the upregulation of several CRKs upon glycan treatment and de novo modelling, suggests that glycans could serve as ligands for CRK receptors. Preliminary data of a glycan array performed on CRK receptors revealed the binding of a CRK with a glycan. In addition to glycans, CRRSPs are also potential regulators of CRK receptors. Preliminary data from immunoprecipitation and mass spectrometry (IP-MS/MS) experiments show that a CRRSP was pulled down with a CRK, supporting the hypothesis that CRRSPs may interact with CRKs. Our findings provide new insights into the regulatory network of CRK receptors.

Poster N° 41

SINGLE CELL ANALYSIS OF THE CLE45-BAM3 PATHWAY IN ROOT DEVELOPMENT

Hang Zhang*, Daria Novikova, Christian S. Hardtke

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

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

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

Poster N° 42

Dissecting the role of CLE40/BAM1 in the Arabidopsis SAM: A story of hormones, small RNAs, and headaches

Meik Thiele*, Svenja Augustin, Rüdiger Simon

A plant's ability to grow indeterminate and modularly relies on apical stem cell pools, termed meristems, which need to be maintained throughout the plant's lifespan. Stem cell homeostasis of the Arabidopsis shoot apical meristem (SAM) is tightly regulated by a negative feedback loop in the center of the meristem, comprising the small secreted signaling peptide CLV3, its receptors (e.g. CLV1), and the homeodomain transcription factor WUS. Close homologs of CLV3 and CLV1, named CLE40 and BAM1, are located in the periphery of the SAM. However, their exact functions are not yet understood. Using confocal microscopy in combination with advanced, quantitative 3D image analysis, we developed a pipeline to precisely study changes in reporter expression profiles within the SAM. Here, we could find remarkable differences in phytohormone signaling activities, in both cle40 and bam1 mutants, compared to wildtypic plants. These findings on the one hand indicate that abundances of specific hormones are changed in these mutants, which will be tested by LC-MS, but also show that the positioning of hormone peaks is affected, suggesting a change in transport. Furthermore, analysis of a degradation-based sensor, reporting the presence of miR171, hints towards a connection between the receptor BAM1 and the mobility of miR171, and potentially other small RNAs, in the SAM. We hypothesize that the CLE40/BAM1 signaling module is involved in different transport processes within the SAM, and thereby fine-tuning stem cell homeostasis.

Poster N° 43

Peptide-receptor signalling pathways directing barley meristem activities

Jan E. Maika*, Isaia Vardanega, Gabriele Buchmann, Maria von Korff, Rüdiger Simon

In plants, inflorescence meristems provide founder cells for the establishment and growth of new organs from a reservoir of stem cells. In Arabidopsis, floral meristems (FM) are directly formed at the flanks of the IM. Grass species, however, produce additional types of meristem, such as the branch meristem (BM), the spikelet pair meristem (SPM) or the spikelet meristem (SM), resulting in more complex inflorescence architectures. Thus the developmental progression and fine-tuned activity of different meristem types in crops is critical for yield production. In some cereals, such as rice (Oryza sativa) and maize (Zea mays), IM size is positively associated with yield traits, while barley (Hordeum vulgare) IM size has a negative correlation with yield traits.

Here, we explore the regulation of meristems and stem cells in barley by leveraging insights from Arabidopsis signalling pathways. Recently it was discovered that signalling pathways similar to the CLAVATA pathway in Arabidopsis also function in barley, controlling the proliferation of various meristems in the barley inflorescence. However, these pathways most probably exhibit unique regulation, expression patterns, and developmental integration specific to grasses. We further investigate the roles of CLE peptides and CLAVATA-family receptor signalling in barley stem cell systems by inducing mutations and generating single-cell RNA sequencing data for above-ground meristem types. Additionally, we will characterize mutants and gene functions through high-throughput RNA in situ hybridisation and reporter lines.

Poster N° 44

Biological functions of patatin-related phospholipase A in plant reproduction and development

Jin Hoon Jang, Hae Seong Seo, Thomas Widiez, **Ok Ran Lee***

Patatin-related phospholipase A (pPLAs) are major lipid acyl hydrolases classified into three groups, and they play diverse roles in plant cellular biology. These roles include regulating cell growth, signal transduction, modulating secondary cell wall composition, inducing haploids, and participating in lipid metabolism. This discussion will focus on the functions of pPLAs in plant reproduction and development. Mutations in the sperm-specific pPLAII, known as MATRILINEAL/NOT LIKE DAD/ZmPHOSPHOLIPASE-A1 (MTL/NLD/ZmPLA1), induce maternal haploids in monocots such as rice, wheat, and foxtail millet. Our study has identified that pPLAIIγ, which is expressed in the Arabidopsis gynoecium, and its homologous gene in rice, pollen-expressed OspPLAIIη, are involved in haploid induction. ZmPLA1-mediated haploid induction is known to result from the accumulation of reactive oxygen species in sperm cells, which leads to chromosome fragmentation and subsequent single fertilization. Additionally, mis-localization of auxin transporters PIN1 and PIN3 may contribute to haploid induction in pplaIIγ mutants. Regarding the plant development, the pPLAIII family of genes seems to play more crucial roles. Overexpression of individual pPLAIII genes leads to a dwarf phenotype and altered cell elongation patterns in several plant species, including Arabidopsis, poplar, rice, and camelina. The pPLAIII overexpression lines exhibit decreased lignin content, a major component of the secondary cell wall, and a significant reduction in the expression of lignin biosynthesis-related genes. Reduced lignin content may be due to disruptions in hydrogen peroxide accumulation or auxin homeostasis. This study will explore the functions of pPLAs in plant reproduction and development, examining their networks and potential underlying mechanisms. By understanding these processes, we can gain insights into the complex roles of pPLAs in plant biology and their implications for agricultural and biotechnological applications.

Poster N° 45

Peptide REF1 is a local wound signal promoting plant regeneration

Wentao Yang*,Huawei Zhai and Chuanyou Li

Plants frequently encounter wounding and have evolved extraordinary regenerative capacity to heal the wounds. Here we report on the characterization of the tomato spr9 mutant that was defective in both, wound-induced defense and regeneration. Gene cloning studies revealed that the SPR9 gene encodes the precursor of SlPep, the single tomato ortholog of the plant elicitor peptide (Pep) family immunomodulatory peptides. We demonstrate that while depletion of the SlPep precursor gene or its receptor gene abolished the regeneration capacity in term of wound-induced callus formation and shoot regeneration, overexpression of these genes led to enhanced regeneration capacity. Moreover, exogenous application of the SlPep peptide dramatically increased regeneration capacity. SlPep was therefore designated as REGENERATION FACTOR1 (REF1). Next, we identified PORK1 is the receptor of REF1.Then we demonstrate that the REF1-PORK1 signaling pathway promotes regeneration through activating master transcriptional factor SlWIND1. Furthermore, SlWIND1 amplifies the REF1 signal through transcriptional activation of the REF1 precursor gene. Thus, REF1 acts as a local wound signal promoting plant regeneration. Since REF1 is broadly conserved in both dicot and monocot plants, application of REF1 dramatically enhanced the transformation efficiency of multiple difficult-to-transform crops, including soybean, wheat, and maize. Discovery of REF1 provides a convenient method to enhance the transformation efficiency of recalcitrant crops by boosting their regeneration capacity.

Poster N° 46

Redox-dependent leucine-rich repeat and cysteine-rich receptor kinases signalling in plants

Ran Lu*, Sergio Martin-Ramirez, Jente Stouthamer, Willy van den Berg, Alejandro Thérèse Navarro, Adam Mott, Elwira Smakowska-Luzan

Reactive Oxygen Species (ROS) are essential signaling molecules in all living organisms, playing a crucial role in plant growth and defense processes. However, the mechanisms by which extracellular ROS are sensed by plasma membrane-localized receptor kinases (RKs) and how they subsequently trigger cellular responses remain largely uncharacterized. Current knowledge suggests that leucine-rich repeat receptor-like kinases (LRR-RLKs) and cysteine-rich receptor kinases (CRKs), two of the largest groups of receptor-like protein kinases in plants, are strongly connected with ROS perception and signaling. Moreover, CRKs possess extracellular cysteine-rich domains that can undergo oxidative modifications, and their expression is generally induced by apoplastic oxidative stress, highlighting them as excellent potential candidates for redox-dependent receptors. These oxidative modifications within the ECDs might alter the structure and consequently modulate CRK-CRK and CRK-LRR interactions. To address this hypothesis, we investigated potential ECD interactions between 40 CRKs and 200 LRRs in No ROS and ROS conditions, using a sensitized high-throughput interaction assay. Obtained redox-dependent CRK-LRR interaction networks were subjected to multilayer filtering by expression pattern, ROS modulation of interactions and presence of oxidative modifications on the CRK ECDs. As a result, CRKs and LRRs form an extensive and intricate heterodimerization network, with its overall architecture significantly altered upon ROS treatment. Therefore, further validation of the CRK-LRR interaction in vitro and in vivo is required, followed by in-depth mechanistic studies to explore ROS-dependent crosstalk among RK families in planta.

Poster N° 47

Identifying immune system components by proximity labelling in tomato

Nicat Cebrailoglu*, Sergio Landeo Villanueva, Christiaan Schol, Matthieu Joosten

Plant immunity is triggered in two ways, in the form of externally (ExTI) or internally (InTI) triggered immunity. External (ExIPs) and internal (InIPs) immunogenic patterns are recognized by receptor-like proteins (RLPs) or receptor-like kinases (RLKs), and nucleotide-binding leucine-rich repeat (NB-LRR) proteins, respectively. Cf-4 is a well-characterized RLP of tomato that recognizes Avr4, an effector of the fungal pathogen Fulvia fulva. Cf-4 lacks a kinase domain and requires the RLK SOBIR1 to initiate downstream signaling, culminating in a hypersensitive response (HR). However, downstream signaling of Cf-4 is still not completely understood and previous findings in Cf-4 transgenic Nicotiana benthamiana should be validated in tomato, the only host of F. fulva. The biotinylating enzyme TurboID can be fused to a protein of interest for detection of close interactors of the bait protein. We generated a SOBIR1-TurboID fusion to identify potential interactors of this co-receptor upon perception of Avr4 by Cf-4 with transmembrane protein Lti6b as negative control. Moneymaker (MM) Cf-4 tomato leaves were infiltrated with either Agrobacterium carrying SOBIR1-TurboID or Lti6b-TurboID, followed by elicitation with Avr4. Thereafter, biotinylated proteins were isolated by streptavidin-based affinity purification and subjected to mass spectrometry. The number of significantly enriched proteins that have a potential function in immunity seriously increased upon the addition of Avr4. Among these, many proteins were previously known to be associated with plant immunity, but some of whose relationship with the Cf4 system was unknown. Interestingly, in addition to known interactor BIR1 (BAK1 interacting RLK1), two novel BIR family members which lack an Arabidopsis ortholog were detected in proximity to SOBIR1. A similar study was performed using BAK1-TurboID which resulted in markedly less enrichment of kinases, but enrichment of known BAK1 interactor BIR2. Markedly, RANGAP2 was enriched significantly both by BAK1 and SOBIR1-TurboID, suggesting an important role for this protein in Cf-4 mediated signaling. Our study highlights the versatility of TurboID-mediated proximity labeling through Agrobacterium-mediated transient expression in tomato for swiftly and specifically determining the potential downstream interactome of plant defense proteins.

Poster N° 48

The secrets of MEMBRANE-ASSOCIATED KINASE REGULATOR 5 in BAM3/CLE45 signaling

Qian Wang* and Christian S. Hardtke.

In Arabidopsis, the BAM3 (BARELY ANY MERISTEM 3)/CLE45 (CLAVATA3/EMBRYO SURROUNDING REGION-RELATED 45) peptide signaling pathway plays an important role in controlling the development of protophloem, which is a key component of the plant's vascular system responsible for the transport of nutrients and signaling molecules. On the one hand, BAM3/CLE45 participates in suppressing ectopic sieve element fate in the neighboring cell files of protophloem. On the other hand, it modulates auxin efflux in the protophloem cell file to precisely control its differentiation timing. Thus, fine-tuned regulation of CLE signaling activity is crucial for proper protophloem development. Our recent findings show that the CLE45 peptide perception is regulated by the apoplastic pH gradient along the phloem cell files. The spatiotemporal signaling compensation and crosstalk between different CLE peptides and receptors ensure the correct formative divisions in the stem cell niche, which are required for the initiation of protophloem. However, the regulatory mechanism at the level of CLE45 signal transduction remains poorly understood. Here, we focus on exploring the function and regulation of a positive regulator in CLE45 signaling transduction, MAKR5 (MEMBRANE-ASSOCIATED KINASE REGULATOR 5), whose loss-of-function mutant is insensitive to CLE45 peptide treatment and can partially rescue the phenotype of the brx mutant, which caused by hyperactive CLE45 signaling. We further analyzed the role of MAKR5 and its homologs through genetic studies and uncovered the mechanism of CLE45-mediated regulation on MAKR5 protein. Our work presents a nice model showing how MAKR5 fine-tunes CLE45 signaling transduction to balance CLE signaling activity.

Poster N° 49

Toward the identification of wound-related signals triggering the transcription factors ERF114 and ERF115 involved in root regeneration.

Daviere Antoine*, Vandendriessche Wiske, Ma Yunjing, Coleman Duncan, Lanssens Fien, De Veylder Lieven.

Compared to animals, plant have evolved remarkable capacities to repair tissues and even regenerate new organs or whole plants upon injury. The ETHYLENE RESPONSE FACTOR 114 (ERF114) and ERF115 play a paramount role in this process enabling tissue repair, callus formation, graft reconnection and root meristem regeneration (Heyman et al., 2013, 2016, 2018). After wounding or cell death induced by DNA damage in the root meristem these transcription factors are induced within hours in the neighboring cells. However, the upstream signals linking wounding to ERFs induction are still unknown. Interestingly, we were able to observe a strong induction of ERF114 in Arabidopsis thaliana upon treatment with exudates collected from wounded plants. Furthermore, wound extracts obtained from thalli of Marchantia polymorpha, a plant with great regeneration skills, were also capable to induce ERF114 in A. thaliana. Using metabolomic analysis and fractionation of the candidates our aim is to identify this putatively conserved wound-related signaling molecule. Next to this untargeted approach, previous works has guided us to hypothesized that pectin fragments called oligogalacturonides (OGs) and known for their role in eliciting plant defense could be involved in ERF114 induction (Canher et al., 2022; Zhang et al., 2022). Indeed, we were able to confirm the inducing capacity of demethylesterified OGs with a degree of polymerization 10 to 15 (DP10-15) on ERF114 and ERF115 whereas shorter DP3 OGs showed no activity. Furthermore, enzymatic fingerprinting of pectins using size exclusion chromatography coupled with mass spectrometry (SEC-MS) (Paterlini et al., 2022) allowed us to observe that wounding caused a reduction in the degree of methylation of root pectins, which can be in favor of OG production. Future investigations will reveal if ERF114 induction in the root following wounding depends on some specific cell wall fragments released upon wounding.

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Poster N° 50

Diversification of the BIR receptor kinase family and its impact on plant health and crop yield

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The intricate interplay between pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) is crucial for plant defense against pathogens. Central to this defense network are the leucine-rich repeat receptor kinase (LRR-RK) pattern recognition receptors (PRR) and its co-receptor BRI1-ASSOCIATED KINASE (BAK1) negatively regulated by the BAK1-INTERACTING RECEPTOR-LIKE KINASE 1 (BIR) proteins. We demonstrated that BIRs play a pivotal role in regulating BAK1 complex formation and cell death. Within the BIR protein family with four members, the two traits i) interaction with BAK1/suppression of BAK1 complex formation and ii) cell death control are developing antagonistically. BIR interactome studies revealed interactions with a nucleotide binding-LRR (NLR) protein CONSTITUTIVE SHADE AVOIDANCE 1 (CSA1) that belongs to the TIR-NLR (TNL) class of ETI receptors. Our findings revealed that CSA1 guards BAK1/BIR3 receptor complexes and is required for the activation of ETI type responses for full immunity. To correlate the antagonistic distribution of traits within the BIR family with their molecular structures, we used alphafold predicted structures. This allowed us to uncover the evolutionary sequence adaptations and structural changes involved in their interactions with both PTI receptors and ETI receptors. We modelled BAK1 BIR CSA1 intracellular interfaces and are currently working on mutating the interacting residues present in the interface to study their impact on cell death control and PTI. In conclusion, our research offers insights into the evolutionary dynamics and functional diversification of the BIR family, providing a foundation for understanding plant immune responses and their implications for crop protection and productivity.

Poster N° 51

A pectin-binding RALF peptide with both a structural and signaling role in the periodic assembly of the plant cell wall

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Secreted Rapid ALkalinization Factor (RALF) peptides have emerged as key components controlling cell wall integrity. However, the inner workings of the RALF pathway remain enigmatic. We show that RALF22, a root hair expressed RALF, has a dual signaling and structural role during cell growth. RALF22 loss-of-function root hairs are short and frequently burst due to a loss of wall integrity. Exogenous RALF22 treatment induces a FERONIA-dependent root hair growth arrest and a FER-independent change in the physicochemical properties of the cell wall. Our data show that this duality is the result of RALF22 interacting with (1) the LLG1/FER transmembrane receptor complex to regulate downstream signaling and (2) the integral cell wall proteins LRX1 and LRX2 to regulate pectic cell wall assembly. In the root hair cell wall, RALF22 forms periodic circumferential rings which colocalize with rings of block-wise demethylated homogalacturonan (HG) and LRX1. Polycationic RALF22 and RALF22-LRX1 bind to and induce the condensation of polyanionic HG in a charge dependent manner.

In vivo, the LLG1-RALF22-FER and RALF22-LRX1-pectin interactions are mutually exclusive. As such, we suggest a model in which FER and LRX1/polyanionic HG compete for RALF22 to sense and regulate pectic cell wall organization, integrity and, as a result, cell growth. Consistent with this model, our most recent findings indicate that FER rapidly senses and responds to changes in the degree of pectin methylesterification in the growing root hair cell wall.

Together, our results reveal a novel mechanism in which RALF22 simultaneously regulates periodic pectin assembly through LRX1/2 and cell wall sensing through LLG1/FER.

Poster Nº 52

Mechanisms underlying the sequestration of TDIF peptides by PXY receptors

Xixi Zhang, Ari Pekka Mähönen

During secondary growth, vascular cambium, a lateral meristem, contains bifacial stem cells that produce secondary xylem on one side and secondary phloem to the opposing side. TDIF-PXY signaling plays crucial role in the maintenance of cambium stem cells (Fisher&Turner 2007; Hirakawa et al.,2008). Recent work by our group and collaborators identified CAMBIUM-EXPRESSED AINTEGUMENTA-LIKE (CAIL) transcription factors as cambium stem cell identity determinants downstream of TDIF-PXY signaling in Arabidopsis roots (Eswaran et al., 2023). Additionally, it was demonstrated that sequestration of phloem-originated TDIF peptides by xylem-expressed PXY is a key component of cambium stem cells positioning mechanism (Eswaran et al., 2023). However, the mechanisms by which PXY proteins sequester TDIF peptides remain unclear. Here we show that TDIF-PXY binding is sufficient to sequester TDIF peptides during cambium activation, regardless of PXY kinase activity. Conversely, PXY kinase activity is necessary to sequester TDIF peptides at the maintenance stage of the cambium. These findings suggest that additional sequestration mechanisms, beyond TDIF-PXY binding, may exist and need further investigation.

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Poster N° 53

Comparative analysis of Arabidopsis thaliana Plant Elicitor Peptides in plant immune responses

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Understanding the perception of external signals at the plasma membrane (PM) and how they influence its structure is crucial for comprehending the PM's role during cellular immune responses. In nature, plants constantly interpret a multitude of environmental cues to adapt and survive amidst various plant pathogens. Pattern recognition receptors (PRRs), embedded within the PM, are essential for sensing both external and internal signals and transmitting them into the cell to elicit appropriate responses. Among these receptors are the PEP RECEPTOR 1 and 2 (PEPR1 and PEPR2) leucine-rich repeat receptor-like kinases, key members of the PRR family. These receptors specialize in recognizing endogenous PEP peptides. On peptide recognition, a signaling cascade activates within the cell, involving various kinases (such as MAPKs), phosphatases and other signaling components like calcium ions and reactive oxygen species (ROS). This cascade leads to a defense response, manifesting as root growth inhibition or deposition of callose and lignin into the cell walls. In this study, we aim to compare the effects of the eight members of the PEP family on late innate immune responses to determine whether these PEPs have redundant functions. We examined the impact of each PEP family member on root growth inhibition and deposition of callose and lignin into the cell walls of Arabidopsis thaliana. Our results demonstrate that all PEP peptides, except PEP3, significantly inhibit root growth in a dose-dependent manner, primarily via the PEPR2 receptor. However, callose and lignin deposition is triggered only by PEP1 and PEP2, with no effect observed from the other PEPs. These findings suggest a possible non-redundancy among members of this endogenous, danger-associated peptide family.

Poster N° 54

A PP2C phosphatase modulates FER activity in the context of the LRX-RALF-FER signaling module

Xiaoyu Hu, Kyle Bender, Gabor Kadler, Shibu Gupta, Anouck Diet, Cyril Zipfel, **Christoph Ringli***

The controlled growth of plant cells requires the coordinated development of the wall surrounding each cell. LRXs (LRR-extensins) play an important role in this process in several ways: They are high-affinity binding site of RALF peptides and LRX-RALF complexes were recently shown to bind pectin in a methylation status-dependent manner which results in compaction of the cell wall (1, 2). In addition, LRXs were shown to be involved in salt-stress tolerance and cell wall integrity (CWI) sensing via the receptor-kinase FERONIA, influencing vacuole development and, thus, cell growth (3, 4). It is not clear to what extent the different functions of LRXs are overlapping and how they execute the structural and the signaling function.

We are investigating the function and signaling role of LRX-related processes by investigating the function of LRX1 of Arabidopsis, a gene that is predominantly expressed in root hairs. lrx1 mutants develop a strong root hair defect which is augmented in the lrx1 lrx2 double mutant where the paralog of LRX1 is also mutated, resulting in a fer mutant-like root hair defect (4). A suppressor screen on the lrx1 mutant resulted in the identification of several rol (repressor of lrx1) mutants, of which rol23 is affected in an H-clade PP2C phosphatase. rol23 not only suppresses lrx1, but also the lrx1lrx2 double mutant and fer-5, a partial loss of function allele of FER. By contrast, the knock-out allele fer-4 is not suppressed by rol23, suggesting that rol23 might require FER protein to exert its suppressive effect. Indeed, different experimental approaches revealed interaction of ROL23 with the cytoplasmic domain of FER, accompanied by a change in the phosphorylation status of FER. Complementation analyses of the rol23 mutant with different PP2Cs revealed specific activity of ROL23 that is shared by PP2Cs of the same clade but not of other clades, revealing specificity of H-clade PP2Cs activity in the regulation of the LRX1-RALF-FER signaling module.

Together, our results suggest that ROL23 is a negative regulator of FER and mutations in rol23 lead to suppression of the lrx1 and fer root hair phenotypes via activating FER (and possibly other downstream signaling components). These findings support our view that LRX proteins function in conjunction with RALF peptides and FER in CWI signaling; a process that involves H-clade PP2Cs as negative regulators.

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Poster N° 55

Exploring the role of peptide hormones in plant growth and fruit development by altering their maturation processes

Carlotta Francese*, Anna Pavanello, Marco Boschin, Marco Armellin, Umberto Salvagnin, Andreas Schaller, Livio Trainotti

Plant growth mechanisms are complex genetically and environmentally determined processes along with fruit development and ripening. The former are fundamental to vegetative growth but also enable the occurrence of the latter. The latter, in turn, are essential for the successful plant reproduction and for economically valuable food sources production. Different plant species share common regulatory networks relying on hormones, TFs, and various signaling molecules. Among these, peptide hormones (PHs), involved in both long and short distance signaling and fulfilling numerous functions (1), are good candidates for regulating different developmental processes.
Recent studies have demonstrated their involvement in different plant processes, but some of their functions, especially in fruit development, are still overlooked. To shed light on this, we took advantage of the evidence that many PH families share common steps in their biosynthetic pathways (2). Therefore, we decided to impair the synthesis of multiple PHs by interfering with the post-translational modifications necessary for their biological activity. Specifically, we focused on two types of post-translational modifying enzymes: proteases of the subtilase family and the Tyrosyl Protein Sulfotransferase (TPST) (3). For the first targets, we employed two different approaches: one based on the overexpression of microbial protease inhibitors (4), and another based on silencing by means of amiRNAs, as for TPST silencing. With the use of different promoters, we impaired PHs synthesis constitutively in tobacco, and in a fruit-specific manner in tomato.
Once the mutant plants were generated, we phenotypically characterized them by examining the overall growth of tobacco, with a focus on the development of specific organs, and by assessing flower and fruit development in tomato. For the tomato plants, we conducted two separate experiments under different conditions: in the first one, we maintained a controlled low flower/fruit load to monitor fruit development and ripening over time without metabolic constrains; in the second, we did not prune the plants to monitor fruit growth among competing sinks. With this approach, we were able to demonstrate the relevance of secreted PHs in many plant processes related to the development of different organs and to prove their important role in the regulatory dynamics occurring in fleshy fruits.
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Poster Nº 56

Novel insights into early responses to RALF perception mechanisms.

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Rapid Alkalinization-Like Factors (RALF) constitute a diverse family of secreted signalling peptides conserved in land plants. RALFs have been extensively studied in the field of plant reproduction, development, and immunity; and their multifaceted roles are increasingly recognized. At the plasma membrane, RALFs are perceived by members of the Catharanthus roseus Receptor-Like Kinase 1-Like family (CrRLK1L) and LORELEI-Like-Glycosylphosphatidylinositol-anchored (LLG) proteins, shaping a complex that initiates RALF-mediated signalling. Different RALFs can induce different physiological responses, with, notably, some RALFs inducing reactive oxygen species (ROS) production, while others instead reducing ROS production triggered by immune elicitors. While the CrRLK1L-LLG module is essential for RALF-induced responses, antagonistic effects underscore the complex nature of RALF signalling. To unravel the intricacies of RALF signalling, we analysed responses induced by different RALFs in different genetic backgrounds. Our findings unveiled rapid physiological changes upon perception of diverse RALFs, including changes in cytosolic calcium concentrations, ROS production and extracellular alkalinization. In addition, we employed proteomic approached to identify potential additional receptor-like kinases involved in RALF signalling. Our results revealed distinct signalling branches for RALF-triggered responses downstream of LLG-CrRLK1L complexes, illustrating the complexity of RALF-induced signalling.

Poster N° 57

Dual function of damage peptide receptor PEPR: promoting both defense and growth during adaptation to phosphate deficiency

Natsuki Tsuchida*, Kota Yamashita, Taishi Umezawa, Yusuke Saijo

Under phosphate (Pi) limitation, plants engage in mutualistic microbes for increasing Pi acquisition, yet mount pathogen resistance via the mechanisms poorly understood. In Arabidopsis thaliana, recognition of damage-inducible Pep peptides by the leucine rich repeat receptor kinases (RKs) PEPR1/PEPR2 leads to activation of defense signaling toward pathogen resistance. We show that defense-related transcriptional responses to Pep1 through PEPRs, but not to bacterial flagellin or fungal chitin, is sensitized in Pi-deprived seedlings. This points to functional diversification of pattern recognition receptors under Pi deficiency. Notably, Pi starvation response (PSR)-related transcriptional reprogramming under low Pi is reduced in non-elicited pepr1 pepr2 plants. Our results suggest that the PEPR pathway not only strengthens defense responses but also contributes to plant growth and may thus be favored during adaptation to phosphate deficiency.

Poster Nº 58

The role of the C-terminal cytoplasmic tail of Cf proteins in the plant immunity

Esranur Budak*, Lisa Van Malssen, Matthieu Joosten

Plants only have an innate immune system to protect themselves against microbial infection. The first layer of defense is mediated by extracellular plasma membrane-associated receptors. These cell surface receptors perceive extracellular immunogenic patterns and trigger the initiation of downstream defense signaling, which finally leads to extracellularly-triggered immunity. Cf resistance proteins of tomato that act against the fully extracellular pathogenic fungus Cladosporium fulvum are so-called receptor-like proteins (RLPs) that localize at the cell surface. Cf proteins require two co-receptors for the activation of downstream cellular responses, because of the lack of a cytoplasmic kinase domain. In the resting state, the Cf protein constitutively interacts with the receptor-like kinase (RLK) SUPPRESSOR OF BIR1 (SOBIR1), whereas upon recognition of the matching effector of C. fulvum by Cf protein. The overall structure of Cf proteins is typical for LRR-RLPs and consists of an LRR ectodomain, an extracellular juxtamembrane domain (eJM), a TM domain and a intracellular juxtamembrane (iJM) domain. The intracellular juxtamembrane domain or C-terminal tail of Cf proteins is rich in basic residues. The juxtamembrane parts of SOBIR1 have opposite charges when compared with the juxtamembrane domains of the various Cf proteins. Possibly, these opposite charges stabilize their interaction. The various Cf proteins have identical C-terminal tails. Cf-4 and Cf-9 have identical iJM domains, and Cf-5 and Cf-2 are also identical for this C-terminal tail. Interestingly, Cf-5/Avr5 and Cf-2/Avr2 triggered response are slow and less strong hypersensitive response than Cf-4/Avr4 and Cf-9/Avr5 triggered response. To understand role of the C-terminal tail of Cf proteins in the intensity of hypersensitive response, the certain domains of Cf-5 and Cf-9 was swapped and checked the effect on the intensity of hypersensitive response. The results suggest that the C-terminal tail of Cf-proteins have a specific role in determining the intensity of the immune response.

Poster Nº 59

Evolution of SERK-mediated signaling in plant development and immunity

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Signal transduction through leucine-rich repeat receptor kinases (LRR-RKs) regulates key aspects of the plant life cycle and stress responses. Most characterized LRR-RKs recruit a co-receptor from the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) subfamily for signaling activation upon ligand perception. SERKs diversified in eudicots, and in Arabidopsis thaliana (At), which has five SERKs, they play overlapping and specific roles. Thus, SERKs likely contribute to LRR-RKs signaling specificity although the molecular basis for such specificity is not fully understood. AtBRI1-ASSOCIATED KINASE 1 (AtBAK1, corresponding to AtSERK3) is the best-characterized SERK member, functioning as co-receptor for many LRR-RKs involved in diverse signaling pathways from development to immunity. Beyond AtBAK1, how other SERKs in A. thaliana or other plant species mediate LRR-RK signaling activation and specificity remains mostly unknown. Through an evolutionary approach, we aim at understanding how LRR-RK/SERK signaling pathways evolved in land plants at the functional and molecular levels, and what the molecular determinants for SERK-mediated signaling and specificity are. Comparing A. thaliana with the evolutionary model system Marchantia polymorpha, which contains only one SERK, we are functionally characterizing a group of LRR-RKs conserved in all land plants and their activation by different SERKs. We generated independent Mpserk mutants which resulted in severe growth defects, suggesting a central role for MpSERK as co-receptor in Marchantia development signaling. In addition, we observed that MpSERK cannot replace AtBAK1's function in reactive oxygen species production during immunity. Conversely, MpSERK can fulfill AtBAK1's role in root development, indicating that certain activation mechanisms are conserved in land plants. Comparative phylogenetic analysis and functional studies employing SERK-RK pairwise combinations will ultimately lead to the identification of the residues responsible for signaling activation and specificity.

Poster N° 60

PvCLE16: a player in coordinating shoot and root responses to soil drought stress

Xinyang Wu, Shiyuan Tao, Ting Sun, Zhuoyi Wang, **Pei Xu***

Plants exhibit a spectrum of responses in both roots and shoots to collectively address soil drought stress, yet the underlying mechanisms that govern the harmonized regulatory network between these above- and below-ground responses are not well understood. In this study, we uncover the pivotal role of a small, mobile peptide, PvCLE16, in orchestrating this intricate process within the common bean. Under standard growth conditions, PvCLE16 is predominantly expressed in the leaves, with only minimal presence in the rapidly growing roots of seedlings. However, under soil drought conditions, the expression of PvCLE16 is significantly upregulated in the leaves, not in the roots, and intriguingly, this upregulation is observed only when soil water content is substantially reduced. This transcriptional enhancement is mediated by the leaf expression of the transcription factor PvTCP10. Through the application of exogenous synthetic PvCLE16 and the overexpression of PvCLE16 in leaves, we demonstrate that PvCLE16 not only induces stomatal closure but also inhibits root elongation. We provide evidence that leaf-derived PvCLE16 can be transported to the roots to exert its functions. Further investigation identified PvBAM3 as the receptor in roots, which leads to the inhibition of root growth by modulating auxin and jasmonic acid signaling pathways. We propose a model wherein the soil drought signal triggers the expression of PvCLE16 in leaves to induce stomatal closure, while the excess PvCLE16 peptide migrates to the roots to repress root growth. The significance of this long-distance peptide movement is hypothesized to serve as a mechanism to prevent excessive root growth inhibition during early drought stress when soil water content is not critically low.

Poster N° 61

Antagonistic Systemin Receptors Integrate the Attenuation with Initiation of Systemic Wound Signaling

Huawei Zhai*, Ke Zhou, Fangming Wu, Lei Deng, Wentao Yang, Chuanyou Li

Plants frequently encounter wounding and have evolved sophisticated mechanisms to activate local and systemic defense responses. A long-standing question is how plants, as sessile organisms, achieve robust wound response without incurring fitness costs resulting from exaggerated defense. Here we report that, two antagonistic receptors, SYR1 and SYR2, of the wound peptide hormone systemin, act in a ligand-concentration-dependent manner to integrate the initiation and attenuation of wound signaling. Whereas SYR1 acts as a high-affinity receptor to initiate systemin signaling through heteromerization with the co-receptor SERK3a, SYR2 functions as a low-affinity receptor to attenuate systemin signaling by out-competing SYR1 for SERK3a binding. The expression of systemin and SYR2, but not SYR1, are up-regulated upon SYR1 activation. Thus, a SYR1–SYR2 negative feedback loop enables plants to integrate the attenuation of systemic wound signaling with its activation. Our findings highlight how plants appropriately respond to wounding based on receptor-mediated calibration of immunomodulatory phytocytokine.

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