5th ZMBP Summer Academy in Plant Molecular Biology

14th - 16th September 2022

Wildberg, Germany

ABSTRACT BOOK

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Molecular Encoding of
Specificity in Plant Processes
SFB 1101

ZMBP

Preface

Welcome to the 5th Summer Academy

It is a pleasure to welcome you to the 5th Summer Academy organized by the Ph.D. students from the Center for Plant Molecular Biology (ZMBP) of the University of Tübingen.

The Summer Academy aims to create a forum for the exchange of ideas, results and discussions between the young researchers themselves and experienced scientists from academia and the private sector in the broad field of basic and applied molecular plant biology. Highlights include talks and student poster presentations as well as workshops addressing topics beyond science, as for instance career prospects for young academics in plant science and beyond.

The talks and posters encompass a wide field of different plant research areas, such as biotic interactions including immunity, cell biology, physiology, development, gene regulation, metabolism and signaling.

Moreover, leisure activities such as hiking in the mountains of the northern Black Forest (don´t forget your rain gear) and barbecue opportunities should not be forgotten, which will facilitate networking even more.

On behalf of the students, I would like to thank the CRC 1101 for the generous financial support of the Summer Academy and the Universitätsbund e.V. of the University of Tübingen for the financing of the very attractive poster awards.

I hope you will enjoy the Summer Academy and your stay in the foothills of the northern Black Forest.

Kind regards

Klaus Harter

(ZMBP)

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Organizing committee

From left to right: Tasnim Zerin, Chaonan Shi, Luiselotte Rausch, Simon Beeh, Ana Andrade, Frank Vogt, Xuan Tran, Niels Gallas

Program Overview

Wednesday, 14.09.2022

Time	Program
10:00	*Arrival*
	Chair: Luiselotte Rausch
10:20	Welcome & Coffee
	Chair: Chaonan Shi *Online Talks*
10:40	Dr. Reinhilde Schoonjans
11:10	Dr. Yanfei Ma
11:40	*Poster Set-up*
12:00	*Lunch*
	Chair: Niels Gallas *Genetics & Gene Regulation*
13:30	Prof. Claude Becker
14:00	Gautier Langin
14:30	*Coffee Break*
	Chair: Tasnim Zerin
15:00	Workshop by Dr. Marc Kuchner
18:00	*Dinner*
19:00	Poster Session I

Thursday, 15.09.2022

Time	Program
07:30	*Breakfast*
08:30	*Hiking/other activity*
12:00	*Lunch*
	Chair: Xuan Tran *Omics, Metabolism & Signaling, Evolution*
13:30	Dr. Philipp Johnen
14:00	Prof. Dietrich Ober
14:30	*Coffee Break*
	Chair: Frank Vogt *Biotic Interactions, Plant Immunity*
15:00	Dr. Honour McCann
15:30	Dr. Ning Zhang
16:00	Dr. Yasin Dagdas
16:30	Prof. Suayb Üstün
17:00	Dr. Farid El Kasmi
17:30	*Dinner/Barbecue*
19:00	Poster Session II

Friday, 16.09.2022

Time	Program
08:00	*Breakfast*
09:00	*Check-out*
	Chair: Simon Beeh *Work in Industry, Development*
10:00	Dr. Juan Suarez
10:30	Dr. Claudia Hener
11:00	Aleksander Parvanov
	Chair: Ana Andrade
11:30	Closing Notes & Awards
12:00	*Lunch*
13:00	*Departure*

Guest Speakers

Emergence and propagation of epigenetic patterns during somaclonal reproduction

J. Isaac Rodríguez[1], Anjar Wibowo[2], Ilja Bezrukov[2], Detlef Weigel[2], CLAUDE BECKER[1]

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Stress and other environmental cues lead to changes at the epigenetic level. While some of these induced changes positively affect the plant's response to a given stress and therefore are of potential interest in increasing stress tolerance, they are usually unstable and get reset during sexual reproduction. Many plants, including several major crops, reproduce or are propagated asexually. We have explored how asexual reproduction influences epigenome conservation and dynamics. In my talk, I will highlight how somaclonal propagation in *Arabidopsis thaliana* enables the reprogramming of the DNA methylation landscape and how these altered methylated states can persist across ensuing sexual reproduction events. We used different genetic backgrounds and various regeneration methods to investigate how these features influence the genetic and epigenetic mutation rates and patterns. Our findings provide insights into the influence of epigenetic variation on somaclonal phenotypic variation in plants.

How proteostasis shapes the plant response to environmental stimuli

Paul Gouguet[1], Gautier Langin[1], Jia Xuan Leong[1], Manuel Gonzalez Fuente[1], Shanshuo Zhu[1], Margot Raffeiner[1], SUAYB ÜSTÜN[1,2]

[1]University of Tübingen, Center for Plant Molecular Biology (ZMBP), 72076 Tübingen, Germany[2]Faculty of Biology & Biotechnology, Ruhr-University Bochum, 44780 Bochum, Germany

Protein homeostasis is epitomized by a tight equilibrium of protein biosynthesis and degradation; the 'life and death' of proteins. Approximately one-third of newly synthesized proteins are degraded. As such, regulated protein turnover is required to maintain cellular integrity and survival. Autophagy and the ubiquitin-proteasome system (UPS) are the two principal intracellular degradation pathways in eukaryotes. Both degradation pathways orchestrate many cellular processes during plant development and upon environmental stimuli. As such, both pathways play a major role during plant-microbe interactions. We have recently identified that autophagy and the proteasome system are exploited by bacterial pathogens to reprogram host cellular pathways. By studying this intimate interplay, we can utilize plant pathogenic bacteria as tools to understand host cellular degradation machineries and to decipher novel components and functions. In my presentation, I will not only cover our recent work on the role of autophagy and the proteasome in plant-microbe interactions but will report on our attempts to identify new autophagy regulators and new functions of known UPS components. I will highlight different examples and discuss our recent advances.

Pathway evolution in plant secondary metabolism – pyrrolizidine alkaloids as model system

DIETRICH OBER

Chemical Ecology and Molecular Evolution
Botanical Institute and Kiel Botanic Gardens
Kiel University, Kiel, Germany

Pyrrolizidine alkaloids (PAs) are a class of secondary compounds that are produced by plants as chemical defense against herbivores. They are part of fascinating interactions between the plant and specialized insects that developed counter adaptations to these plant toxins. Within the angiosperms, several plant lineages are described to produce PAs. Studies on the first pathway specific enzyme, i.e., homospermidine synthase, have shown that the pathway evolved several times independently during angiosperm evolution. Therefore, this system is a promising tool to study the evolution of pathways in plant secondary metabolism by comparative approaches. Recent results will be presented that on the strategies of gene identification, on models of gene evolution, and on some unexpected observations resulting from protein characterization.

Dynamic signaling systems in plant shoot growth

YANFEI MA[1, #], Jan U. Lohmann[1]

[1]Department of Stem Cell Biology, Centre for Organismal Studies, Heidelberg University; D-69120 Heidelberg, Germany
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Unlike animals, plants specified by postembryonic growth need to initiate organs continually, which is driven by robust stem cell systems. In the shoot, however, stem cell robustness faces fluctuation from a dynamic signalling of auxin, a potent phytohormone for differentiation. Here, we find that stem cells are not susceptible to auxin triggered differentiation in present of WUSCHEL, a core transcription factor for stem cell identity. WUS repressively binds to most auxin signalling components and response genes in such way as to restrict signalling output. However, a basal level of signalling is still required for stem cell activity. Finally, we demonstrate that to gate auxin output, WUS acts via regulating histone acetylation at target loci. We illuminate a developmental dynamic of how to keep stem cells close to differentiating cells undifferentiated involves a single master regulator mediated transcription network on cell differentiation program.

Site and Mode of Action Identification and Characterization of Early Herbicidal Leads

PHILIPP JOHNEN[1]

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Understanding the site and mode of action of a herbicide is key for its efficient development, the evaluation of its toxicological risk, efficient weed control and resistance management. Therefore, a robust procedure for the identification thereof is key for the efficient development of new crop protection agents.

After lead identification in the green house, we routinely use a combination of physionomics, metabolomics, chemo-proteomics and biochemical assays to exclude unwanted and identify new sites of action (SoAs) of herbicidal compounds. This setup led to the identification of acyl-acyl carrier protein (ACP) thioesterases (FATs) as the SoA of cinmethylin, a pre-emergence herbicide for the control of cool season grasses in cereals (1). Analysis of the downstream effects caused by cinmethylin treatment indicated that the class of FATs is indeed the predominant SoA *in planta*. Knowledge of FATs as the target of cinmethylin led to the identification of a hidden FAT inhibitor cluster among commercially available herbicides and showed the unique characteristic of cinmethylin in terms of wheat selectivity (2, 3). Furthermore, identification of a diverse set of FAT inhibitors provides a new tool set to study the role FATs in plant growth and development.

1. Campe R, Hollenbach E, Kammerer L, Hendriks J, Höffken HW, Kraus H, et al. A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis. Pestic Biochem Physiol. 2018;148:116-25.
2. Johnen P, Zimmermann S, Betz M, Hendriks J, Zimmermann A, Marnet M, et al. Inhibition of acyl-ACP thioesterase as site of action of the commercial herbicides cumyluron, oxaziclomefone, bromobutide, methyldymron and tebutam. Pest Manag Sci. 2022.
3. Brabham C, Johnen P, Hendriks J, M. B, Zimmermann A, Gollihue J, et al. Herbicide symptomology and the mechanism of action of methiozolin. Weed Science. 2020(16):18–30.

GM Plant risk assessment at the European Food Safety Authority

REINHILDE SCHOONJANS[1], Andrea Gennaro[1], Tommaso Raffaello[1].

[1]European Food Safety Authority, Parma, Italy

The GMO Panel provides independent scientific advice on the safety of genetically-modified plants. This advice is used by the Risk Managers (the Members States and the European Commission) to grant market authorisation to the GMO or not. The Panel is supported by EFSA staff, Working Group experts, contractors (ISA) and a network of Member States representatives. During the work EFSA engages with stakeholders following the GMO Legal frameworks and Transparency Regulation. The Risk Assessment of plants entails multiple investigations to be engaged on a case-by case basis depending on the Crop/Trait/Use combination and grouped as follows:

- Food/feed safety assessment based on toxicology, immunology, allergenicity, human/animal nutrition, dietary exposure, biochemistry and metabolism, food chemistry, compositional analysis statistics and field trial design, animal feeding trials
- Environmental risk assessment based on plant ecology/biology/agronomy/pathology/physiology, ecotoxicology, insect ecology and population dynamics, impact on non-target organisms (entomology), ecosystem services, soil science (soil organisms, biochemical processes, abiotic processes), gene flow, persistence and invasiveness (weediness), experimental design (field trial and laboratory test.
- Molecular characterisation of the GM Plant based on molecular biology and characterisation of genetic transformation techniques, genetic stability and plant breeding/physiology, bioinformatics, proteomics, transcriptomics, genomics, mathematical modelling, horizontal gene transfer, antibiotic resistance, expression analysis, protein analysis.

Immune signalling of intracellular plant immune receptors

FARID EL KASMI[1]

[1]ZMBP. University of Tübingen, Tübingen, Germany

Perception of potential microbial phyto-pathogens happens first at the plasma membrane by cell-surface pattern-recognition receptors (PRRs) that initiate a wide range of immune responses. Adapted pathogens, however, have evolved effector proteins to dampen this PRR-triggered immunity. In resistant plants these effectors are recognized by intracellular nucleotide-binding leucine-rich repeat receptors (sensor NLRs) and NLR activation results in the re-initiation of PRR-triggered immune outputs and often in the programmed like cell death of the infected cell. Many sensor NLRs and also some PRRs require the presence of a conserved immune signalling convergence hub formed by the plant-specific lipase like proteins EDS1, PAD4 and SAG101 and a small NLR subfamily – the RNLs – for proper immune activation. Recently we showed that RNLs localize and function at the plasma membrane in a phospholipid-dependent manner. This is in accordance with the findings that RNLs and other cell death inducing NLRs form oligomeric 'resistosomes' at the plasma membrane, which function as cation channels. I will summarize our contribution to the current model of NLR function and also give an overview of the recent breakthrough findings and still open questions of the field of plant NLR biology.

Leveraging evolutionary diversity to discover new autophagy mechanisms in plants and humans

YASIN DAGDAS

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Vienna BioCenter (VBC), Vienna, Austria

Selective autophagy is a highly conserved quality control mechanism that remodels cytoplasmic contents during cellular reprogramming. It is induced by a wide spectrum of biotic and abiotic stress responses to keep the cytoplasm in tune with the changing environment. Selective nature of the process is ensured by selective autophagy receptors (SARs) that bridge the cargo with the core autophagy machinery to mediate selective cargo degradation. SARs recruit the autophagy machinery through interacting with ATG8, a ubiquitin-like protein conjugated to the phagophore. Despite recent advances in metazoans, the catalogue of SARs, therefore our understanding of autophagy mediated cellular quality control, is incomplete in plants. Here, we leverage a state-of-the-art proteomics approach to identify novel autophagy players conserved across land plants. Unlike conventional proteomics approaches, we devised a simple, high-throughput approach to identify new cargo receptors and validated them using *in vivo* and *in vitro* approaches. I will present our latest findings on the mode of action of one of these cargo receptors that turned out to be also conserved in humans. Altogether our findings highlight the power of leveraging evolutionary diversity to discover new autophagy pathways across the eukaryotes.

Student speakers

Genetics & Gene Regulation

The proteasome regulatory feedback loop coordinates photosynthetic proteins homeostasis

GAUTIER LANGIN[1], David Biermann[1], Daniela Spninti[2], Mirita Franz-Wachtel[3], Boris Macek[3], Fredirick Börnke[2], Suayib Üstün[1,4]

[1] Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany
[2] Leibniz-Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany.
[3] Interfaculty Institute for Cell Biology, Department of Quantitative Proteomics, University of Tübingen, Tübingen, Germany.
[4] Faculty of Biology & Biotechnology, Ruhr-University Bochum, Bochum, Germany.

The 26S proteasome (Ptsm) complex is the major degradation center of many subcellular proteome. Due to this large quantity of substrates, the Ptsm needs to be tightly regulated to ensure correct protein turn-over and avoid proteotoxic stress. In eukaryotes, this regulation is mediated through a negative feedback loop, where functionally conserved transcription factors are substrates and transcriptional activators of the Ptsm. In plant, NAC53 and NAC78 have been shown to mediate activation of Ptsm genes during chemical inhibition. However, there is no evidence for their proteasomal degradation. Furthermore, the impact of such loop on other biological process remains elusive. Using multidisciplinary approaches, we confirmed the presence of a Ptsm regulatory feedback loop in Arabidopsis. We identified NAC53/78 as substrates of the ER-associated degradation pathway. Analyzing Arabidopsis transcriptome, we found Ptsm genes up-regulation happens in concert with down-regulation photosynthesis associated nuclear genes (PhANGs). We showed PhANGs expression to be coordinated by the Ptsm regulatory feedback loop, where NAC53/78 actively repressed PhANGs expression binding to a similar cis-element they bind on Ptsm genes promoter. Our work highlights the Ptsm regulatory feedback loop in plant and propose how this feedback loop is involved in the regulation of another key process, photosynthesis.

From stem cells through differentiation: landmarks of the re-wiring cistrome

ALEKSANDAR P. PARVANOV[1], Tom Denyer[1], Marja C.P. Timmermans[1]

[1]ZMBP, University of Tübingen, Germany

In multicellular life, irrespective of look, type, or function, every cell emerges from a stem cell. But, the similarity between a stem cell and its own progeny is limited, and even more so between sibling progenitors committing towards distinct cell-type lineages.

Utilising the power of high-throughput single-cell technologies, I measure transcriptome and chromatin dynamics in post-embryonic root development with the aim to describe the intracellular events happening along lineage commitment and differentiation.

Posters

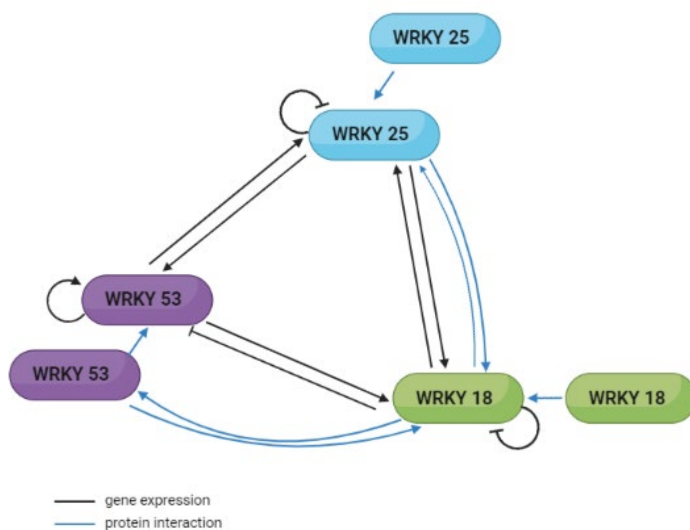
Genetics & Gene Regulation

Molecular Modulation of the WRKY 53, WRKY18 and WRKY 25 regulatory subnetwork of leaf senescence

ANA G. ANDRADE[1], Jasmin Doll[1], Ulrich Zentgraf[1]

[1] Center for Plant Molecular Biology (ZMBP), Tübingen, Germany

Plant senescence is an important developmental process with a great impact on plant productivity. It involves a very complex molecular regulatory network in which two transcription factor families play a key role, the WRKYs, and the NACs. In Arabidopsis, among the WRKY family, WRKY53 has been shown that acts in a hub position of the regulatory network. Interestingly, W53 harbors in its promoter 3 W-boxes meaning that could be regulated by other WRKYs. For instance, it has been shown that WRKY18 is a negative upstream regulator while W25 acts as a positive upstream regulator. In addition, both are downstream targets, and both are able to interact at the protein level with WRKY53 [1] [2]. Although new insights facilitate the understanding of this subnetwork, it remains unknown how this is regulated at the genetic level. Thus, we try to decipher the molecular mechanisms driving the interactions within the W18/W25/W53 by dissecting the different modules of the proteins. For example, by creating gene deletion and chimeric constructs of W18/W25, we try to uncover the domains that specify these interactions. Our approaches include protein-protein and DNA-protein interaction analysis, transactivation, and phenotypic analysis of the plants. Preliminary results show that mainly the N-terminal domain of W18 is involved in the repression of W53. Also, the deletion of either WRKY-domain (1) or WRKY-domain (2) converts W25 into a repressor of PW53. All this has given us an insight that the specificity of this complex subnetwork is closely related to the WRKY domain presence and the capacity to regulate their promoters.



Model of the WRKY18-53-25 subnetwork. Black arrows describe the effects on gene expression, and blue arrows show the protein-protein interaction. W18 is a negative upstream regulator of W25 and W53 and a downstream target of W53. In contrast, W25 is a positive upstream regulator but is also a downstream target of W53. In addition, all members of this network can form homo and heterodimers.

References:
[1] J. Doll *et al.*, 2020, Frontiers in Plant Science
[2] M. Potschin *et al.*, 2013, Journal of Plant Growth Regulation

Fatty acid modifications modulating Arabidopsis NLR cell death function

SIMON BEEH[1], Sruthi Sunil[1], Luisa Teasdale[2], Detlef Weigel[2], Farid El Kasmi[1]

[1]ZMBP, Tübingen, Germany
[2]Max Plank Institute for Biology, Tübingen, Germany

Plant immune responses mediated by intracellular NLR immune receptors usually comprise a hypersensitive response (HR)-like programmed cell death. Recent structural studies of phylogenetically distinct CC-type NLR suggest a common mechanism of cell death induction. Oligomerized CC domains penetrate the plasma membrane and form ion channels, eventually leading to HR (Wang *et al.*, 2019; Jacob *et al. 2021;* Bi *et al.*, 2021*).* We identified the truncated Arabidopsis CC-type NLR PM5, conserved in several *Brassicaceae* species, that lacks most of the CC domain and that induces cell death ectopically when overexpressed. PM5 belongs to a phylogenetic group of NLRs that possess predicted N-terminal fatty acid post-translational modification (PTM) sites, important for cell death function and localization and implicate constitutive membrane association (Qi *et al.,* 2012; Sunil *et al.*, unpublished data). Our goal is to identify the mechanisms behind these PTMs and to determine how these NLRs trigger cell death even though their CC domain might be attached to the inner leaflet of cellular membrane compartments.

Bi, G. *et al.* (2021), *Cell*, doi: 10.1016/j.cell.2021.05.003.
Jacob, P. *et al.* (2021), *Science*, doi: 10.1126/science.abg7917.
Sunil, S., *et al*. (unpublished data)
Wang, J. *et al.* (2019), *Science*, doi: 10.1126/science.aav5870.
Qi, D. *et al*. (2012), *Plant Physiology* doi: 10.1104/pp.112.194035.

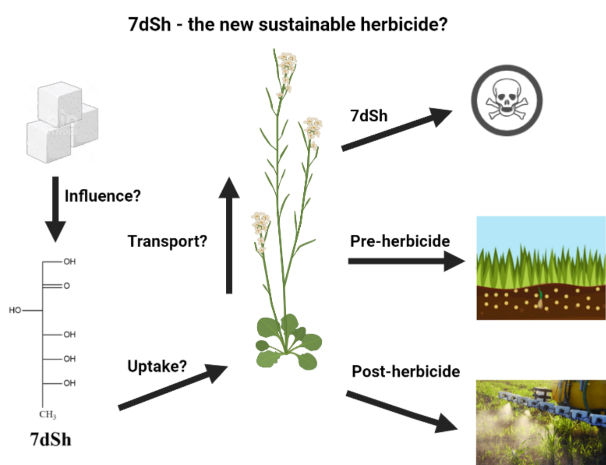
Omics, Metabolism & Signaling

Investigation of the Inhibitory effect of 7dSh on the Shikimate Pathway in plants

MARVIN BRAUN[1], Svenja C. Saile[1], Sabine Hummel[1], Torren Bischoff[1], Joachim Kilian[1], Klaus Harter[1], Üner Kolukisaoglu[1]

[1] Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany

Long-term food security requires efficient agricultural management that leads to an increase in yields. The required high yields can only be achieved by minimizing the growth of competing vegetation (weeds), among other things. Today, this is predominantly achieved through the usage of herbicides. Glyphosate, currently the most widely known and used herbicide, is becoming increasingly controversial due to possible side effects on humans and the increasing incidence of resistant weeds. Therefore, more environmentally friendly and sustainable alternatives are needed. The biochemical agent 7dSh (7-deoxysedoheptulose) acts as a competitive inhibitor of the enzyme DHQS (3-Dehydroquinate synthase) in the shikimate pathway, which plays a central role in the biosynthesis of aromatic amino acids. Interestingly, the deoxy sugar exhibits herbicidal activity on *Arabidopsis thaliana* and other plant species by inhibiting seed germination. However, for the development of a more environmentally compatible herbicide, the mode of action of 7dSh needs to be investigated in more detail. We use mainly metabolomics and transcriptomic studies to uncover the molecular and physiological consequences of the application of 7dSh in plants. Additionally, we analyze whether and how 7dSh can be used as an herbicide both before and after weed germination. Using GC-MS, we could show that 7dSh is taken up by the weeds *Arabidopsis thaliana* and *Abutilon theophrasti*, laying the foundation for further metabolic studies in the shikimate pathway. In another approach, we investigated the impact of different DHQS isoforms on the shikimate pathway. Preliminary results point to an alternative, non-canonical, route to produce shikimic acid in plants.



Mechanistic insights into cell death execution by Bs4C, a Xanthomonas TALE-inducible executor resistance protein from pepper

DANALYN R. HOLMES[1], Robert Morbitzer[1], Kaltra Xhelilaj[1], Julien Gronnier[1], Johannes Stuttman[2], Thomas Lahaye[2]

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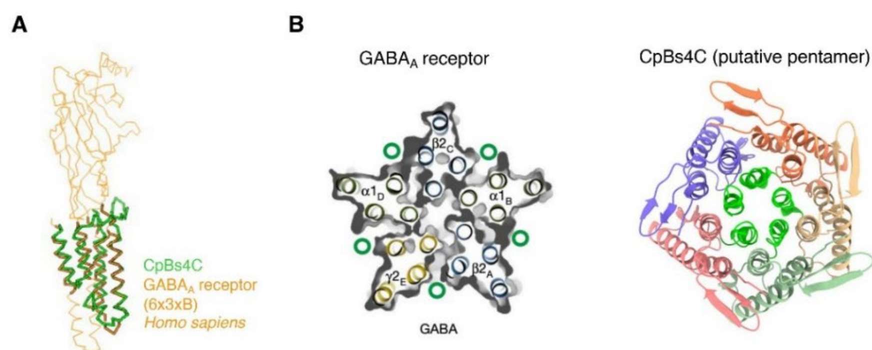
[2]Department of Genetics, Institute for Biology, Martin-Luther University, Halle, Germany

The phytopathogenic bacteria *Xanthomonas euvesicatoria* (*Xe*) preys on agriculturally relevant pepper plants by injecting effectors into host cells to promote pathogen virulence. *Xe* injects transcription activator like effector (TALE) proteins, whereby incompatible hosts activate resistance genes (R genes). One of these TALEs, AvrBs4, activates executor R gene Bs4C in *Capsicum pubescens* (CpBs4C) and induces cell death (Strauß et al., 2012). Homologues of CpBs4C have been found in most solanaceous species, some of which are not natural hosts of *Xe* and all of which do not have an AvrBs4 effector binding element (EBE) in their promoter, aside from CpBs4C. All of the identified homologues have a similar topological structure to that of CpBs4C and they all localize in a similar fashion. Moreover, CpBs4C presents strong structural similarity to that of neurotransmitter receptors coming from *H. sapiens*, *M. musculus*, and *T. californica*. Utilising these characterised receptors as structural and functional inspiration, we found that the CpBs4C homologues self-associate and form a complex. Taken together, the fact that CpBs4C homologues are present in many non-natural hosts of *Xe*, and they have striking similarity to a protein class of known function in another kingdom could lead us toward the true origin of CpBs4C and its original function.



***Capsicum pubescens* Bs4C has strong structural homology with neurotransmitter receptors.**
(A) Structure of the transmembrane domains of CpBs4C (in green) overlaid with the GABA$_A$ receptor, from *Homo sapiens*.
(B) The pentameric structure of the GABA$_A$ receptor, taken from Kim et al., 2020 (left panel), compared with the putative pentamer of CpBs4C transmemrane domains (right panel).

Cell Biology & Physiology

The plant specific NRL (NPH3/RPT2 Like) protein family: Identification of spatially distinct in vivo interaction partners of NPH3 using proximity labelling

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Center of Plant Molecular Biology (ZMBP), University of Tübingen

NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) is a key component of the blue light (BL) induced phototropic response, acting in concert with phot1 and RPT2 at the plasma membrane (PM). Upon BL perception, phot1 mediated phosphorylation of the third last residue of NPH3 (Ser744) induces 14-3-3 protein association, triggering PM detachment followed by NPH3 condensates formation in the cytosol. Reset of darkness induces NPH3 relocation to PM (Reuter et al. 2021; Sullivan et al. 2021). Yet, the biochemical role of NPH3 remains elusive. We aim to identify NPH3 protein interactions partners upon its different subcellular localizations using proximity labelling (TurboID) to understand its functions. We have generated a library of NPH3 variants fused to TurboID (YFP:TurboID:NPH3). Our results confirmed specific biotinylation of 14-3-3 proteins only upon co-expression with YFP:TurboID:NPH3. Experiments in Arabidopsis transgenic lines showed differential biotinylation patterns of BL-irradiated samples as compared to dark adapted plants, suggesting distinctive interaction partners triggered by the different light conditions. Next steps include IP-LC/MS for further analysis and identification. In addition, we focused on other NRL members by studying a conserved binding motif for 14-3-3 proteins (C- terminus). Our results indicate that DOT3, RPT2 and ENP (also known as MAB4/NPY1) can interact with 14-3-3 proteins.

References:

Reuter, L. et al. 2021. *Nature Communications* 12(1): 1–15.

Sullivan, S. et al. 2021. *Nature Communications* 12(1).

A host transcription factor plays a central role in target gene activation by Xanthomonas and Ralstonia TALE-like proteins

NIELS GALLAS[1], Robert Morbitzer[1], Markus Wunderlich[1], Edda von Roepenack-Lahaye[1] & Thomas Lahaye[1]

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During infection, **t**ranscription **a**ctivator-**l**ike **e**ffectors (TALEs) from plant-pathogenic *Xanthomonas* are injected into host cells. Inside the plant cell, TALEs specifically activate target genes by binding in their promoters. One class of these targets are resistance genes (*R*-genes). *R*-genes often encode so called executor proteins that lead to a local cell death reaction, providing resistance. In pepper plants (*Capsicum annuum*) the TALE AvrBs3 activates the executor *R*-gene *Bs3*. The total landscape of TALE-dependent virulence and avirulence is still poorly understood. To uncover components needed for Bs3-dependent cell death we analyzed a population of EMS-mutagenized pepper plants and screened for absence of AvrBs3-induced cell death. Mutant line #1231 did not show AvrBs3-dependent cell death and displayed a SNP in the CDS of TFIIAγ, changing a single amino acid (tfiiAγ^{D42N}). *TFIIAγ* encodes a general transcription factor and member of the RNA-polymerase II complex. In rice (*Oryza sativa*), TFIIAγ is known as Xa5 and was previously described as needed for TALE-induced target gene activation. Here, we now describe how tfiiAγ^{D42N} found in pepper line #1231 affects virulence and avirulence of TALE-carrying bacteria. We also show that tfiiAγ^{D42N} does not only affect *Xanthomonas* TALE target gene activation but also RipTALs, TALE-like proteins from *Ralstonia solanacearum*.

Control of cell wall properties is required for stem cell maintenance in the Arabidopsis shoot apical meristem

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Stem cells in the shoot apical meristem (SAM) are maintained by the transcription factor WUSCHEL (WUS) and are characterized by CLV3 expression. Upon division, stem cell daughter cells are displaced from the meristem centre and pass through several transcriptionally distinct domains undergoing specific differentiation program. Examining available cell type-specific expression data suggests tight control of cell wall properties; specifically, expression of PMEs (Pectin methylesterases, a class of cell wall modifying enzymes) is very low in the stem cell (pCLV3) domain compared to peripheral SAM regions, whereas pectin biosynthetic and catabolic enzymes are generally expressed uniformly in the SAM. Previously, a ChIP-seq data revealed that many SAM-expressed PMEs are targets of WUS peaks. By using a cell type-specific expression toolkit, that allows the expression of PMEs in a systematic way in different SAM domains, we also found that, stem cell expression of PMEs negatively affects stem cell identity and reduces the size of the stem cell domain within 48 hours of induction, i.e. before a significant number of cells are displaced into the SAM periphery. Thus, we hypothesize that the key SAM regulator WUS has a significant role in maintaining stem cell wall properties and cell wall remodelling promotes differentiation, thereby controlling cell fate via feedback regulation. We aim to decipher how feedback signaling from the cell wall affects cell identity in the SAM. Furthermore, we want to uncouple remodelling gene expression from WUS control and determine the function of cell wall feedback signalling in cell identity maintenance.

Cell Biology & Physiology

Phosphoinositide phosphates dependent regulation of Microtubules dynamic by MDP25 in A. thaliana pavement cells

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The plasma membrane (PM) is at the interface between cell wall and microtubule (MT) arrays which makes it a key compartment to understand how cells transduce external stimuli. While perception of chemical components is well characterized, little is known about how plant cells respond to mechanical signal. However, it has been proposed such transduction to involve cytoskeleton through a direct link with the PM. To decipher this interplay, we combined phosphoinositide phosphates (PIPs) alteration, mechanical stress and analysis of pavement cells microtubules. We identified the tubulin- and PIPs-binding protein MICROTUBULE DESTABILIZING PROTEIN 25 (MDP25), as a key component of this interplay. We showed PIPs perturbation to strongly impact microtubules dynamic. We could show MDP25 is a regulator of the MT response during PIPs alteration. Furthermore, we found MDP25 as a positive regulator of MT reorganization during mechanical stress (MS). Performing an IP-MS/MS, we found this protein to display a diverse interactome including cytoskeletons and trafficking components. We showed MDP25 associates with MT and actin cytoskeletons at specific regions. Altogether, our data demonstrate MDP25 mediates fine microtubule regulation at the interface between PM and cytoskeleton during mechanical stress and highlighted novel components as putative regulators.

Cell Biology & Physiology

Dark side of the membrane – how hidden tracks reveal new hormone receptor dynamics

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Brassinosteroids (BRs) regulate various processes in plants such as cell elongation, growth and overall development. To understand cell elongation initiation on the molecular level, precise investigation of BR sensing and signal transduction are of utmost interest. The BR receptor Brassinosteroid Insensitive 1 (BRI1) induces a fast response at the plasma membrane (PM), leading to the onset of cell elongation. To enable this fast BR response, the components are organized in distinct subcompartments (nanodomains). Their organization and composition are assumed to be closely related to signalling specificity and integration.

We aim to investigate the spatiotemporal behaviour of BRI1 and BR signalling-associated proteins using single particle tracking with photoactivated localization microscopy (sptPALM). Thereby we track individual fluorophore-tagged proteins in the PM to characterize diffusion properties and the formation of protein clusters *in vivo*. This gives access to previously unavailable parameters potentially relevant for growth hormone perception and cell physiological output, that can be integrated into the computational model of this pathway.

Our objective is to elucidate underlying concepts of signalling complex formation within the heterogeneous environment of the PM. The acquired knowledge about this signalling module is extendable to other perception systems and thereby contributes to overall understanding of nanodomains and signalling.

Cell Biology & Physiology

Deciphering RLP44-linked LRR-receptor dynamics and signaling specificity at the plasma membrane

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Integration of external signals with intrinsic developmental programs are of vast importance for plants and rely on cell surface receptors located at the plasma membrane. The best characterized and most common group are the leucin-rich-repeat receptor-like kinases (LRR-RLKs). They built a complex network with LRR-RLPs which resemble LRR-RLKs but lack a kinase domain. The extensive sharing of components between different pathways with specific signalling outputs, raises the question of how distinct signalling responses can be achieved. Mechanisms such as spatial separation, post-translational modification or regulated trafficking are known to play an important role. However, it is unclear how these processes intersect to spatially and temporally modulate plasma membrane receptor dynamics. Recently, RLP44 was identified as a cell wall-binding leucin-rich repeat receptor-like protein that interacts with two different receptor complexes, namely BRASSINOSTEROID INSENSITIVE 1 (BRI1) and PHYTOSULFOKINE RECEPTOR1 (PSKR1). Both share BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (BAK1) as co-receptor. Since the constitution of the signalling modules is well described we are now interested in dynamic parameters (diffusion coefficient, cluster sizes, resident time) and how these are influenced. Here, we make use of advanced microscope techniques, basically Variable-angle total internal reflection fluorescence microscopy-based single particle tracking photoactivated localization microscopy (VA-TIRF sptPALM).

Exploring the functional diversification of the C4 proteins encoded by bipartite begomoviruses and the correlation between C4 proteins and resistance breakdown in plants

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Geminiviruses, which are among the most devasting plant pathogens, are a family of circular single-strand(ss) DNA viruses; begomovirus is the largest genus within this family. Begomovirus includes monopartite begomoviruses (with only one genomic component) and bipartite begomoviruses (with two genomic components), and encode 6-8 proteins. Among those proteins, C4 is the smallest and most diverse protein. However, the overview of the functional diversity of C4 is still not clear. C4 encoded by the begomovirus tomato yellow leaf curl virus (TYLCV) shows plasma membrane (PM), chloroplast, and plasmodesmata (PD) localization. Chloroplast-localized C4 and PD-localized C4 interfere with SA-mediated defense and RNA interference movement, respectively. An N-terminal myristoylation site and a chloroplast transit peptide (cTP) are essential for C4 to localize at the PM and chloroplasts, respectively. To test the function of C4 proteins with different combinations of presence/absence of the N-myristoylation motif and the cTP, here we chose the C4 proteins from the bipartite begomoviruses African cassava mosaic virus (ACMV), bean golden mosaic virus (BGMV), East African cassava mosaic virus (EACMV) and tomato yellow leaf curl virus-Mild (TYLCV-Mild). BGMV C4, EACMV C4 and TYLCV-Mild C4 have both the myristoylation site and the cTP, while AVMC C4 does not. Our results show that the C4 proteins from different geminiviruses show diverse subcellular localizations, suggesting that C4 proteins may have different functions or target the same plant pathway in different subcellular compartments. Highlighting the potential relevance of C4 for the viral infection, TYLCV-Mild C4 is essential for TYLCV-Mild to breakdown the resistance to Tomato yellow leaf curl disease (TYLCD) in a tomato wild cultivar, but this is not accomplished by TYLCV C4; the molecular, cellular, and functional differences between these two C4 proteins will also be investigated as part of this project. In the future, we expect unveil the functional portfolio of geminiviral C4 proteins and identify plant processes convergently targeted by these positional homologues.

Plant biochemistry

Unravelling the molecular mechanisms underlying the replication of geminiviruses

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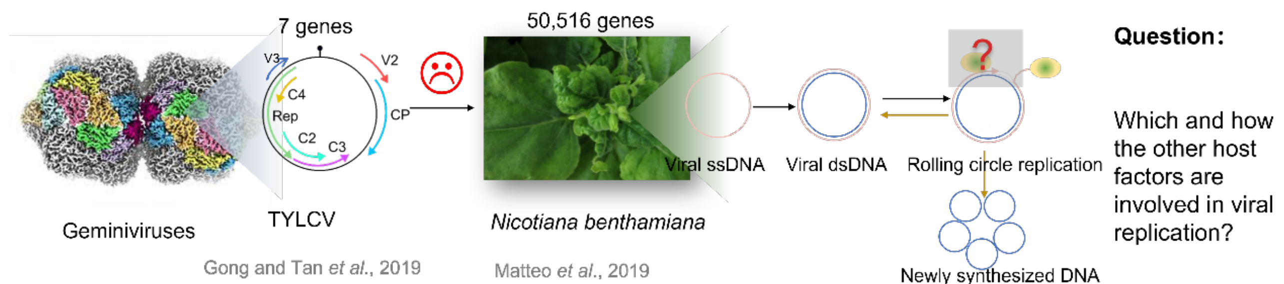
Geminiviruses are a family of plant viruses characterized by twin icosahedral capsids and circular, single-stranded DNA genomes. They cause devastating diseases in crops around the world. Replication of geminiviruses is the first step of the viral cycle. We have recently shown that DNA polymerase α and δ are required for viral replication. However, the composition of the viral replisome remains mostly elusive. The viral Replication initiation protein (Rep) is highly conserved, and is the only viral protein essential for replication. Here, we use Rep from tomato yellow leaf curl virus (TYLCV) as a bait to capture host factors involved in viral DNA replication in *Nicotiana benthamiana* via TurboID-based proximity labelling (PL) followed by mass spectrometry (MS) analysis. The PL-MS data have uncovered several known replication-associated proteins in proximity to Rep. In addition, this approach has unveiled splicing as a process required for viral replication. Currently, we are exploring the putative function of the selecting protein candidates in the viral replication process.



Cell Biology & Physiology

Investigation of calcium-dependent phosphorylation of the transcription factor bZIP63 by Ca^{2+} dependent protein kinase 3 (CPK3)

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The bZIP transcription factor (TF) family consists of more than 70 known members in *Arabidopsis thaliana*, subdivided in 13 groups. The DNA- binding domain (DBD) of these TFs is highly conserved within the subgroups leaving room for specific regulation, for instance via phosphorylation. The phosphorylation of specific residues may influence various molecular properties of the TF, such as subcellular localisation or DNA-binding.

bZIP63 is part of the C-group of the bZIP TF- family and was suggested to play a role in the plant energy metabolism, seed maturation and germination. Upon energy deprivation the kinase SnRK1 phosphorylates bZIP63 and promotes its heterodimerisation with the S1-group member bZIP11. It was shown before, that crude extract from *Arabidopsis thaliana* phosphorylates bZIP63 in a calcium-dependent manner. By performing an in-gel kinase assay followed by LC-MS/MS a member of the CPK family CPK3 was identified amongst others [Mair et al., 2015, eLife]. The elimination of 8 preliminarily identified CPK3 candidate phosphorylation sites outside of the DBD of bZIP63 showed a remaining calcium- dependent phosphorylation. This hints to a CPK3-dependent regulation within the DBD.

My current work is the identification of the specific target site(s) of CPK3 within the little studied DBD of bZIP63. We are using a phos-tag-gel based approach to detect the phosphorylation state of transiently expressed DBD variants in the presence of active and inactive CPK3 *in planta*. The identification of these residues and the deciphering of their role for bZIP63 molecular properties may lead us to unravel new functions and pathways of the bZIP63 and bring us one step closer to the understanding of specific gene regulation.

Studies of a putative peptidoglycan layer in the envelope of vascular plant chloroplasts

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Eukaryotic cells are able to turn sunlight into chemical energy. This reaction takes place in organelles called chloroplasts, which originated from former free-living cyanobacteria and were acquired in an endosymbiotic event.

The bacterial peptidoglycan (PGN) cell wall layer, important for protection and cell division, consists of sugars and amino acids. It is believed that chloroplasts lost this cell wall layer in the course of evolution. Newest research showed that non-vascular plants like *Physcomitrella patens* still possess peptidoglycan [1] and relevant PGN biosynthesis genes are still present in the genome of land plants [2]. The question is whether vascular plant chloroplasts also possess a PGN layer.

Different methods were used to investigate chloroplasts of *Arabidopsis thaliana* and *Nicotiana benthamiana*. Physiological growth experiments with PGN targeting antibiotics showed seedling growth inhibition. PGN biosynthesis KO mutants had a defect in chloroplast division. *In vivo* labeling of PGN revealed a fluorescing layer around chloroplasts and *in vitro* biochemical binding assays showed an interaction of isolated plant PGN and PGN recognizing proteins.

In contrast to the prevailing opinion, these are independent lines of evidence for the presence of peptidoglycan in vascular plant chloroplasts.

References:

[1] T. Hirano *et al.*, 2016, The Plant Cell
[2] M. J. van Baren *et al.*, 2016, BMC Genomics

Characterization of Arabidopsis helper NLR regulators

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Plants evolved a two-layered immune system to defend themselves against pathogens. In the first layer, plasma membrane localized pattern recognition receptors (PRRs) perceive pathogen-associated molecular patterns (PAMPs) and subsequently associate with co-receptors to initiate PAMP-triggered immunity (PTI). In the second layer, intracellular nucleotide binding-leucine rich repeat proteins (NLRs) bind pathogen-derived effector molecules and activate effector-triggered immunity (ETI). Many NLRs require a NLR subfamily, the RPW8-like coiled coil NLRs (RNLs) which are also termed helper NLRs, for signalling. In *A. thaliana*, there are two RNL subfamilies, the NRG1s and the ADR1s. Expression of the autoactive mutant of ADR1-L2, ADR1-L2^{D484V}, induces a characteristic autoimmune phenotype. Recently, the PRR co-receptor BKK1 was discovered to be a positive and specific regulator of ADR1-L2^{D484V} activity as the autoimmune phenotype of ADR1-L2^{D484V} is suppressed by the loss-of-function allele of *BKK1*, *bkk1-1*. This project aims to elucidate the mechanism by which BKK1 regulates the ADR1-L2^{D484V} induced autoimmune phenotype, and potentially RNL-mediated disease resistance.

Battle between bacterial effector and plant autophagy

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Plant pathogenic effectors perturb the proteolytic pathways autophagy and ubiquitin-proteasome system (UPS), disrupting host cellular processes to enhance virulence. We study the bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria (Xcv)* which is a significant agricultural pest. Results from our lab show that *Xcv* subverts host autophagy during infection. We identified XopL as an effector with a role in this subversion of host autophagy. We showed that XopL interacts with and ubiquitinates a component of the host autophagic machinery, SH3P2. This results in SH3P2 degradation via the proteasome, which leads to higher *Xcv* bacterial growth. In turn, the plant defends itself using defence-related selective autophagy receptor NBR1. We showed that NBR1 is upregulated and accumulates during coexpression with XopL, colocalizes with XopL in aggregates, is present in a complex with XopL, and causes the autophagic degradation of XopL. Knockdown of NBR1 also resulted in higher *Xcv* bacterial growth. To provide further mechanistic insight, NBR1 mutants lacking ubiquitin-binding domains were tested for interaction with XopL. We found that ubiquitin-dependent and -independent mechanisms drive this interaction. Our study is a novel example in plants of “effectorphagy”, where selective autophagy targets bacterial effectors, and further gives mechanistic insight behind this process.

Cell Biology & Physiology

The HIR protein family and nanoscale organization of receptors in plasma membranes

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Plants possess a number of defense mechanisms to protect themselves against various pathogens. Recognition of pathogens is carried out by intra- and extracellular receptors. Transmembrane receptors at the plasma membrane are located in distinct membrane nanodomains. Stomatin, prohibitin, flotillin, and HflK/C (SPFH)-domain containing proteins have been demonstrated to play a role in the formation of membrane subdomains. Hypersensitive-induced reaction proteins (HIRs) belong to the SPFH protein family and are enriched in membrane domains. They are induced after pathogen treatment and *hir2* mutants show an altered defense response. Additionally, HIRs have been identified as interactors of numerous plasma membrane receptors, such as Brassinosteroid associated kinase 1 interacting receptors (BIR). BIRs negatively regulate pattern recognition receptor complex formation with the co-receptor BAK1.

The aim of this work is to generate an integrated model of the HIR protein function with focus on their impact on membrane nanodomain formation, receptor localization and defense response in plants. To achieve this, we will test single and multiple *hir* mutants in pathogen assays, study their effect on BAK1 complex formation and BAK1-related signaling pathways. Single-particle tracking photoactivated localization microscopy (sptPALM) will be applied to study nanodomain formation and receptor dynamics in the *hir* mutant background.

Exploring the functional diversification of the C4 proteins from monopartite begomoviruses and curtoviruses and investigating the different viral infection strategies between TYLCCNV and TLCYnV

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Among all the six to eight proteins encoded by geminiviruses, devastating plant pathogens worldwide, C4 is the most functionally diverse one. It has been proven that C4 is essential for viral full infectivity in all geminiviruses tested to date. C4 is also described as a symptom determinant. However, the diversity in subcellular localization and biological function of C4 proteins encoded as well as the underlying molecular determinants and mechanisms are not clear. Here, we investigate the subcellular localization and functional diversity of C4 proteins encoded by a selection of monopartite begomoviruses, belonging to the largest genus within the geminivirus family, by confocal microscopy and functional assays. We will define the interactome of these selected C4 proteins by TurboID-based proximity labelling (PL). We found that the selected C4 proteins show different subcellular localization, and some re-localize between subcellular compartments (plasma membrane to chloroplasts) upon exogenous treatments with the bacterial elicitor peptide flg22 or the plant peptide Pep1. Currently, we are exploring the potential function of selected C4 proteins in suppression of silencing, suppression of SA-mediated defences, and cell cycle activation, as well as their impact on plant development. Ultimately, we expect to integrate the obtained information on subcellular localization, biological function, and interacting partners, in order to uncover crucial biological processes manipulated by these viral proteins.

Towards an atlas of REMORIN-nanodomains associated functions

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Plasma membrane lipids and proteins are dynamically organized into diverse nano-environment giving rise to fluid molecular patchworks also referred as nanodomains (Gronnier *et al*., 2018, *Trends in Plant Science;* Jaillaise*., et al 2020, Plant Physiology*). REMORINs (REMs) constitute a plant specific protein family which recently emerged as regulatory components of immunity, symbiosis, and development (Gouguet *et al*., 2021, *Plant Physiology*). REMs of different groups tend to form distinct and coexisting NDs which are proposed to host specific signalling pathways (Jarsch *et al*., 2014, Plant Cell). However, the molecular functions associated with REMs nanodomains largely unknown. To answer this question, we will perform an organism-wide functional characterization of REMs nanodomains architecture, composition, and function. Using live cell imaging approaches and super resolution microscopy, we are characterizing time-resolved and context-depended expression and nanodomain organization of REMs. To identify function associated with distinct REM nanodomains we are coupling single cell transcriptomic and proteomic approaches. Finally, in order to genetically dissect REM NDs associated functions, we are generating a collection of REM mutants using a multiplexed CRISPR strategy. Our project will shed light on REMORIN-mediated regulation of cell surface signalling across different cell types and tissues.

Cell Biology & Physiology, Plant Immunity

Arabidopsis thaliana ADR1 ectopic activity is regulated by inter- and intrafamilial interactions with ADR1-L1, ADR1-L2 and NRG1.1.

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Helper NLRs function as downstream signaling partners to other (sensor) NLRs in the perception of pathogen effectors. In *Arabidopsis thaliana*, two families of helper NLRs are present: the ADR1 family and the NRG1 family. We identified and investigated a constitutive PR1 expression (*cpr*)-like phenotype, specific to the *adr1-l1 adr1-l2 nrg1.1* mutant. Removing *ADR1* suppressed the *cpr*-like phenotype, suggesting that ADR1 causes this phenotype in absence of ADR1-L1, ADR1-L2 and NRG1.1. Furthermore, we showed this phenotype requires EDS1, PAD4 and SID2. Overexpression of ADR1 induces cell death and autoimmunity. Co-infiltrating *ADR1* with *NRG1.1* suppressed ADR1 cell death activity. However, co-infiltration of *ADR1* with *ADR1-L1* or *ADR1-L2* does not, which may contrast the suppression of the auto-immune phenotype in *A. thaliana* by ADR1-L1 and ADR1-L2. *NRG1.2* did not suppress the ADR1-induced cell death in *N. benthamiana*, in line with our observations in *A. thaliana*. Since the ADR1-induced cell death was EDS1-independent, we are currently investigating whether ADR1 overexpression activates immune signaling in *N. benthamiana* and whether this immune signaling is suppressed by ADR1-L1, ADR1-L2, NRG1.1 or even NRG1.2. Through these efforts, we aim to propose a model how helper NLRs interact and regulate each other during immunity.

Biotic interaction

A TIR-NBS-LRR protein is necessary for BAK1 autoimmune phenotypes and links BAK1-mediated cell death to effector triggered immunity

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The membrane-localized co-receptor BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE 1 (BAK1/SERK3), regulates different signaling pathways including growth and development, immune response by directly interacting with and positively regulating multiple ligand binding receptors. The BAK1-interacting RK BIR3 can prevent BAK1-ligand binding receptor interaction by directly interacting with both ligand-binding receptors and BAK1 (and all members of the SERK family). The interactome of BIR3 revealed a BIR3 interacting TIR-NBS-LRR (TNL) protein CONSTITUTIVE SHADE AVOIDANCE 1 (CSA1). Double mutants in *bir3 bak1* show enhanced cell death compared to *bak1* single mutants. Our investigations revealed that mutations in *csa1 suppress bir3 bak1*-mediated cell death, suggesting that CSA1 guards the integrity of the BAK1 BIR3 complex. *csa1* mutants are more susceptible to *Pto* DC3000 hrcC, a bacterium that triggers defense responses only via MAMPs. MAMP inducible cell death triggered by pg23, a peptide that initiates PTI via RLP 42, is mediated by CSA1 independent of typical PTI responses. PTI-independent contribution of CSA1 to plant immunity shows that both PTI and ETI responses are activated downstream of BAK1/BIR3 complexes for full plant immune responses.

Development

Deciphering the role of RLP-mediated cell wall sensing in plant development

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Monitoring the cell wall state is a crucial part of plant development as the cell wall is involved in all of plant cell growth and remodeling. Recently the conserved RECEPTOR-LIKE PROTEIN 44 in the family of plant RLPs has been shown to be required for cell wall homeostasis, particullarly in pectate limiting conditions.1 In addition, RLP44 is shown to act in Brassinosteroid (BR) and Phytosulfokine (PSK) signaling through direct interaction with RECEPTOR-LIKE KINASE complexes BRI1/BAK1 and PSKR1/BAK1 potentially serving as a mediator between signaling pathways.2 Confocal microscopy and Co-immunoprecipitation (Co-IP) reveal that a pectate binding deficient RLP44 mutant has lower steady-state levels at the plasma membrane, likely due to a higher endocytosis rate. Loss of RLP44 pectate binding leads to incomplete ability to maintain regular root growth in pectate limiting conditions consistent with decreased BRI1 interaction in Co-IP. Finally, we show that RLP44 is necessary for the BR transcriptional response in a protoplast Luciferase assay.

1. Wolf, S. et al., 2014, PNAS
2. Holzwart, E. et al., 2018, PNAS

Cell Biology & Physiology

Deciphering the role of RLP-mediated cell wall sensing in plant development

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

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