# ZMBP Summer Academy 2022

## Student Abstracts

Genetics & Gene Regulation

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

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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.

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

Biotic Interactions

# Fatty acid modifications modulating Arabidopsis NLR cell death function

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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.

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Omics, Metabolism & Signaling

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

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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.

Biotic Interactions

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

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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.

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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ABSTRACT
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.
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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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Abstracts
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.

Biotic Interactions

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

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During infection, transcription activator-like effectors (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 TFIIAg, changing a single amino acid (tfiiAg$^{D42N}$). TFIIAg encodes a general transcription factor and member of the RNA-polymerase II complex. In rice (Oryza sativa), TFIIAg is known as Xa5 and was previously described as needed for TALE-induced target gene activation. Here, we now describe how tfiiAg$^{D42N}$ found in pepper line #1231 affects virulence and avirulence of TALE-carrying bacteria. We also show that tfiiAg$^{D42N}$ does not only affect Xanthomonas TALE target gene activation but also RipTALs, TALE-like proteins from Ralstonia solanacearum.

Genetics & Gene Regulation

# The Proteasome Regulatory Feedback Loop Coordinates Photosynthetic Proteins Homeostasis

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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 binds 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.

Field-of-Research (Development)

# Deciphering specificity of MYB transcription factors during root barrier formation

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Over the course of their evolution, plants have developed mechanical barriers to defend themselves from environmental threats. During early development in roots, this defensive role is fulfilled by the epidermis, along with the endodermis or exodermis. Upon organ secondary/radial growth, this role is assumed by the periderm that consists of 3 layers: the phellogen, which forms the suberized phellem and the parenchyma-like phelloderm.
While several studies focused on the mechanisms behind the development of other meristematic tissues and defensive barriers, the molecular mechanisms underlying periderm development are mostly unexplored. Previous studies identified members of the MYB84 transcription factor subclade as periderm regulators. These TFs regulate both phellogen proliferation and suberin deposition in the phellem (Wunderling et al., 2018, New Phytologist) (Molina et al. unpublished), however how they act at molecular level and whether they work in other developmental contexts, is largely unknown.
Within this project, I will investigate whether the TFs of the MYB84-subclade have functional specificity across root barriers and meristems, their tissue-specific transcriptional responses, and whether they form protein complexes with themselves or with proteins from other families.
This current study will uncover the mechanisms behind stem cell differentiation processes and shed more light into periderm development.

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.

Biotic Interactions

# 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.

Development & Gene Regulation

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

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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.

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 achived. 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).

Biotic Interactions

# 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 virsues; 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.

Development

# Single-cell analysis of the transcriptome of shoot apical meristem and leaf primordia of *A. thaliana*

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The shoot apical meristem (SAM) is a precisely-regulated tissue which gives birth to all above-ground plant organs following germination. The SAM fundamentally comprises stem cells residing at its apex, which proliferate and acquire diverse, specific identities upon sequential rounds of division. Stem cells are surrounded by other specialized cell types which help to orchestrate this delicate and dynamic process. We have used single-cell RNA-sequencing (scRNA-Seq) to reveal cell-type-specific expression patterns of key regulators of the SAM with an emphasis on leaf primordia development, and the tissue differentiation therein. Another intriguing event on which we focus is the establishment of early vascular precursors. We have constructed and annotated a scRNA-Seq atlas of 40,000 high-quality cells, incorporating all major shoot tissues including rare populations within the SAM. We have identified early-tissue cell clusters and have reconstructed developmental trajectories describing the progression of young leaf primordia. These allow us to track, at high resolution, specific events responsible for organ development. Further, we have also taken advantage of the single cell resolution to reconstruct tissue-specific gene regulatory networks (GRNs). These methods allow us to improve our understanding of key processes in the early stages of plant life, as well as those occurring in already differentiated tissues.

Cell Biology & Physiology

# Investigation of calcium- dependent phosphorylation of the transcription factor bZIP63 by Ca2+ 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.

Development

# Getting into shape - Identification of novel factors guiding the morphogenesis of dumbbell-shaped guard cells in grasses

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Stomata are epidermal valves at the leaf surface that open and close to absorb photosynthetic carbon dioxide and to restrict water loss through transpiration. While stomata in Arabidopsis consist of two kidney-shaped guard cells (GCs) that surround a pore, grasses form morphologically innovative stomata, consisting of two dumbbell-shaped GCs flanked by two lateral subsidiary cells (SCs). This “graminoid” morphology is associated with faster stomatal movements and contributes to more water use-efficient gas exchange. In the model grass Brachypodium, the last step of GC morphogenesis is guided by the bHLH transcription factor BdFAMA that is expressed after the symmetric division of the guard mother cell (GMC) until full maturation. The stomatal bdfama mutant shows undifferentiated paired GCs that do not mature into the characteristic dumbbell shape and do not form a pore resulting in seedling lethality. To identify novel players that guide the morphogenesis of the dumbbell-shaped GCs specific to grasses, we made use of transcriptomics and forward genetics approaches. First, we performed comparative RNA-seq of wt and fama mutant developmental leaf zones. Second, we used cell typespecific and single-cell RNA-seq of sorted GCs expressing a fluorescent BdFAMA reporter gene. These datasets yielded several (GC-specific) candidate genes that are differentially expressed in the fama background and that might have a role in the differentiation and morphogenesis of functional GCs. We are currently generating and analyzing mutant and reporter lines of some of these candidates. In conclusion, comparative transcriptomics and genetic approaches will yield novel factors that shape the unique dumbbell GCs found only in the grass family. Keywords: stomata; grasses; development; guard cells; scRNA-seq;

Cell Biology & Physiology

# 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

Biotic Interactions

# 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.

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.

Biotic Interactions

# 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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ABSTRACT
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.

Omics, Metabolism & Signalling

# 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 signaling 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.