SFB924

Plant Biology of the Next Generation

- **Conference of the SFB924**
  "Molecular mechanisms regulating yield and yield stability in plants."
- **October 11 – 13, 2017**
  Wissenschaftszentrum Weihenstephan
  Technical University of Munich
- **Registration (free)/conference office**
  www.sfb924.wzw.tum.de

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HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

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Invited speakers

- **Frederic Berger** (Gregor Mendel Institute, Vienna, AT)
- **Tom Brutnell** (Danforth Center, St. Louis, MO, USA)
- **Idan Efroni** (Hebrew University of Jerusalem, Rehovot, ISR)
- **Hiroo Fukuda** (University of Tokyo, Tokyo, JP)
- **Daniel Gibbs** (University of Birmingham, Birmingham, UK)
- **Rita Groß-Hardt** (University of Bremen, Bremen, DE)
- **Caroline Gutjahr** (Ludwig-Maximillians-University, Martinsried, DE)
- **Ulrich Z. Hammes** (University of Regensburg, Regensburg, DE)
- **Erika Isono** (University of Constance, Constance, DE)
- **Regine Kahmann** (Max-Planck-Institute for Terrestrial Microbiology, Marburg, DE)
- **Eric Kemen** (Max Planck Institute for Plant Breeding Research, Cologne, DE)
- **Maria von Korff** (University of Düsseldorf, Düsseldorf, DE)
- **Olivier Loudet** (INRA, Versailles, FR)
- **Klaus Mayer** (Helmholtz Zentrum, Munich-Neuherberg, DE)
- **Dave Nelson** (University of California, Riverside, USA)
- **Thomas Ott** (University of Freiburg, Freiburg, DE)
- **Francois Parcy** (Biosciences and Bio-technology Institute of Grenoble, Grenoble, FR)
- **Holger Puchta** (Karlsruhe Institute of Technology, Karlsruhe, DE)
- **Stefanie Rosa** (University of Potsdam, Potsdam, DE)
- **Klaus Schmidt** (KWS Saat, Einbeck, DE)
- **Kay Schneitz** (Technical University of Munich, Freising, DE)
- **Thorsten Schnurrbusch** (IPK Gatersleben, Gatersleben, DE)
- **Ken Shirasu** (RIKEN Centre, Yokohama, JP)
- **Uwe Sonnewald** (University of Erlangen-Nürnberg, Erlangen, DE)
- **Stefanie Sprunck** (University of Regensburg, Regensburg, DE)
- **Corina Vlot-Schuster** (Helmholtz Zentrum, Munich-Neuherberg, DE)
- **Dolf Weijers** (University of Wageningen, Wageningen, NL)
- **Phil Wigge** (The Sainsbury Laboratory, Cambridge, UK)
- **Cyril Zipfel** (The Sainsbury Laboratory, Norwich, UK)

Conference venue

Lecture hall 21/Hörsaal 21

Wissenschaftszentrum Weihenstephan

Technische Universität München

Hans-Carl-von-Carlowitz Platz 2

WLAN

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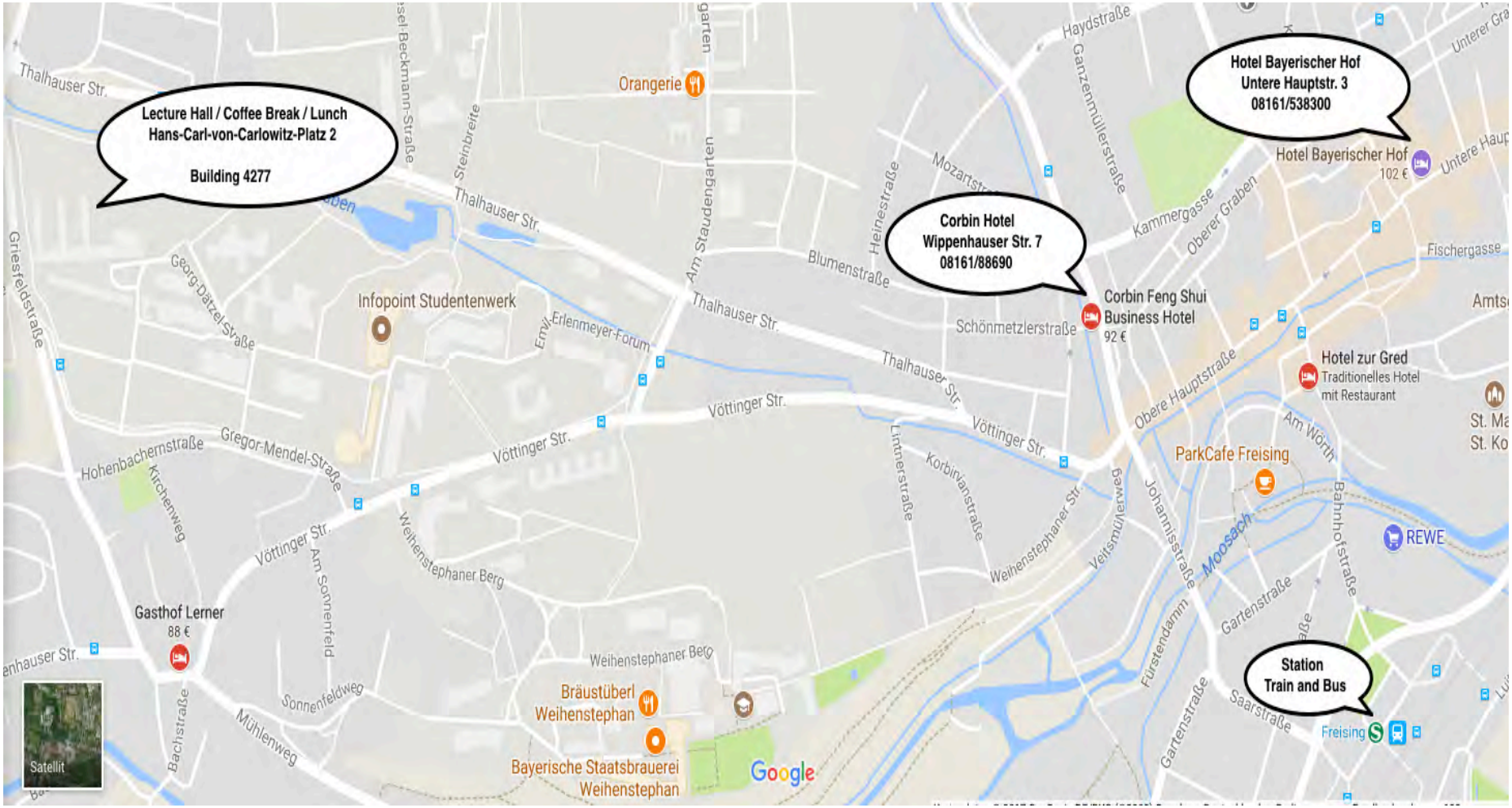
Travel expenses

Invited speakers can download the travel expenses claims form here: http://sfb924.wzw.tum.de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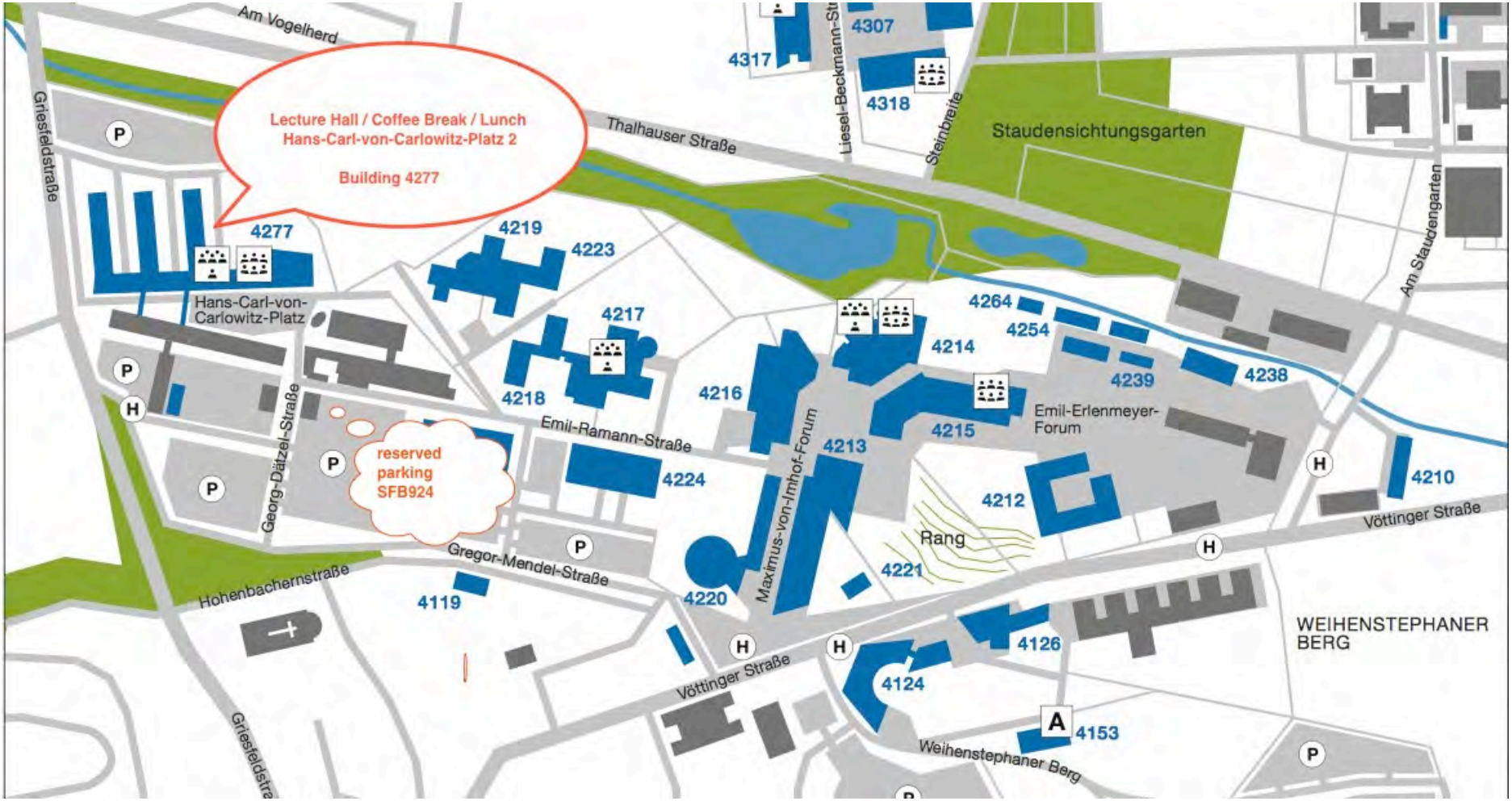
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4153
Weihenstephaner Berg

Program

Wednesday October 11, 2017

12:00 - Registration *(small snacks will be served)*

Session 1 Chair: Kay Schneitz (TUM)

14:00 [T1.1] Dolf Weijers (University of Wageningen, NL)

Origin and evolution of the nuclear auxin response system

14:30 [T1.2] Idan Efroni (Hebrew University of Jerusalem, ISR)

De novo initiation of meristems in Arabidopsis and tomato

15:00 [T1.3] Hiroo Fukuda (University of Tokyo, JP)

The VISUAL system visualizes key regulators of early vascular development

15:30 *Coffee break and posters*

Session 2 Chair: Karina van der Linde (University Regensburg)

16:30 [T2.1] David Nelson (University of California-Riverside, CA, USA)

The evolution of strigolactone and karrikin receptors in plants with specialized germination strategies

17:00 [T2.2] Caroline Gutjahr (LMU, Munich, DE)

Cross kingdom lipid transfer from plants to arbuscular mycorrhiza fungi

17:30 [T2.3] Phil Wigge (The Sainsbury Laboratory, Cambridge, UK)

Temperature sensing and signal integration in plant development

18:00 [T2.4] Daniel Gibbs (University of Birmingham, UK)

Control of VRN2 stability by the N-end rule pathway links oxygen sensing to PRC2 function in flowering plants

18:30 [T2.5] Erika Isono (University of Constance, DE)

Regulation of ubiquitin-dependent intracellular membrane transport processes in plants

afterwards *Mixer with drinks, cool beer, food and posters*

Thursday **October 12, 2017**

Session 3 **Chair: Ulrich Hammes (TUM)**

9:00 **[T3.1] Uwe Sonnewald (University of Erlangen-Nürnberg, DE)**

Unraveling the protein composition of plasmodesmata of Arabidopsis leaves

9:30 **[T3.2] Kay Schneitz (TUM, Freising, DE)**

The atypical Arabidopsis GPI-anchored β-1,3 glucanase ZERZAUST controls floral organ development mediated by the atypical receptor-like kinase STRUBBELIG

10:00 **[T3.3] Francois Parcy (CNRS Grenoble, FR)**

A structural journey among floral regulators

10:30 **[T3.4] Thorsten Schnurrbusch (IPK Gatersleben, DE)**

Genetic regulation of spike form and grain number in temperate cereals

11:00 *Coffee break and posters*

Session 4 **Chair: Thomas Ott (University of Freiburg)**

11:30 **[T4.1] Ulrich Hammes (TUM, Freising, DE)**

Exporters – the missing link in long-distance transport of amino acids

12:00 **[T4.2] Rita Groß-Hardt (University of Bremen, DE)**

Building and bypassing plant polyspermy barriers

12:30 **[T4.3] Stefanie Sprunck (University of Regensburg, DE)**

Molecular and cellular events during flowering plant gamete interactions

13:00 *Lunch break and posters*

(*Thursday* *October 12, 2017*)

Session 5 **Chair: Chris-Carolin Schön (TUM)**

14:00 **[T5.1] Klaus Schmidt (KWS Saat, Einbeck, DE)**

Improvement of wheat in the era of New Breeding Methods

14:30 **[T5.2] Tom Brutnell (Danforth Center, St. Louis, USA)**

Dissecting C4 photosynthetic pathways in the grasses; from comparative genomics to functional genomics

15:00 **[T5.3] Maria von Korff (University of Düsseldorf, DE)**

Genetic control of spikelet development in barley

15:30 *Coffee break and posters*

Session 6 **Chair: Thomas Dresselhaus (University Regensburg)**

16:00 **[T6.1] Frederic Berger (Gregor Mendel Institute, Wien, AT)**

Maintenance of Polycomb gene silencing depends on H3 variants in plants

16:30 **[T6.2] Holger Puchta (Karlsruhe Institute of Technology, DE)**

Genome engineering in plants: Past, Present, Future

17:00 **[T6.3] Olivier Loudet (INRA, Versailles, FR)**

Natural variation for growth response to the environment in Arabidopsis

17:30 **[T6.4] Maximilian Griesmann (Helmholtz Zentrum Munich, DE)**

Deciphering the nitrogen-fixing root nodule symbioses with comparative phylogenomics

ca. 18:15 *Departure by bus from the conference for Munich and*

Conference dinner at the Hofbräuhaus München

Return ca. 23:00

Friday **October 13, 2017**

Session 7 **Chair: Stefanie Ranf (TUM)**

9:00 **[T7.1] Regine Kahmann**

(Max-Planck-Institute for Terrestrial Microbiology, Marburg, DE)

Core effectors in smut fungi: an amazing treasure box

9:30 **[T7.2] Eric Kemen**

(Max-Planck-Institute for Plant Breeding Research, Cologne, DE)

Leaf microbial communities: structure and dynamics

10:00 **[T7.3] Thomas Ott (University of Freiburg, DE)**

Dynamic patterning of cell surface receptors and partners

10:30 **[T7.4] Martin Parniske (LMU, München, DE)**

A novel component of the CCaMK/CYCLOPS complex regulates root nodule symbiosis

11:00 **Coffee break**

Session 8 **Chair: Caroline Gutjahr (TUM)**

11:30 **[T8.1] Cyril Zipfel (The Sainsbury Laboratory, Norwich, UK)**

Regulation of receptor kinase-mediated immune signaling

12:00 **[T8.2] Corina Vlot-Schuster (Helmholtz Zentrum Munich, DE)**

Monoterpenes promote systemic acquired resistance within and between plants

12:30 **[T8.3] Stefanie Ranf (TUM, Freising, DE)**

Immune sensing of lipopolysaccharide in animals and plants: same but different

13:00 **Claus Schwechheimer (TUM, Freising, DE)**

Concluding remarks and end of conference

Talk abstracts

[T1.1] Origin and evolution of the nuclear auxin response system

Sumanth K. Mutte[1], Hirotaka Kato[1], Carl Rothfels[2], Michael Melkonian[3], Gane Ka-Shu Wong[4], and **Dolf Weijers**[1]

[1]Laboratory of Biochemistry, Wageningen University [2]Department of Integrative Biology, University of California, Berkeley, CA, United States of America; [3]Botanical Institute, Cologne Biocenter, University of Cologne, Germany; [4]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; BGI-Shenzhen, Bei Shan Industrial Zone, Yantian District, Shenzhen, China

The small signaling molecule auxin controls numerous developmental processes in land plants, acting mostly by regulating gene expression. Auxin response proteins are represented by large families of diverse functions, but neither their origin nor the evolution of diversity is understood. We have used a deep phylogenomics approach to reconstruct both the origin and the evolutionary trajectory of all nuclear auxin response protein families. We found that, while all subdomains found in auxin response proteins are ancient, a complete auxin response mechanism is limited to land plants. Functional phylogenomics predicts defined steps in the evolution of response system properties. We have performed a comparative transcriptome analysis across six ancient lineages of charophytic green algae, bryophytes and ferns and show how these innovations have shaped a sophisticated response mechanism. We discovered the existence of a mechanistically independent transcriptional auxin response system in green algae. Finally, genetic analysis in the liverwort *Marchantia polymorpha* revealed unexpected contributions of ancient non-canonical proteins in auxin response as well as auxin-unrelated function of core transcription factors. Our study provides a functional evolutionary framework for understanding diverse functions of the auxin signal.

[T1.2] *De novo* initiation of meristems in *Arabidopsis* and tomato

Chen Yahav, Michal Lieberman-Lazarovich and **Idan Efroni**

The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University, Rehovot, Israel

Plant growth is mediated by the activities of regions of patterning and proliferation termed meristems, which house and maintain the stem cells. The initial meristems are formed during embryogenesis, but plants are able to recruit differentiated cells to form meristems, de novo, throughout their lives. This capacity underlies plants' remarkable developmental plasticity and ability to recover from severe injury.

We employed lineage tracing and single-cell RNA-Seq to study the regeneration of roots after excision of their stem cell niche and showed that the root tip regenerates by formation of a new meristem from remnant tissues in the stump (1). Spatial dynamics during early stages of the meristem initiation were similar to the embryogenesis, and differed from those apparent during meristem maintenance, suggesting that meristem initiation and maintenance follows different developmental paths (2).

To test the universality of these results and its application to non-wound induced meristem initiations events, we turned to tomato (*Solanum lycopersicum*) stem-borne roots. Tomato is unique in its ability to naturally generate roots *de novo* along large and easily accessible stems, making it a promising model system to study meristem initiation. By developing new tools for imaging of auxin response, we discover that early stages of tomato stem-borne root initiation are characterized by auxin-cytokinin dynamics that are similar to those observed during root tip regeneration. Curiously, our analysis has also uncovered an unexpected induction of ordered cellular proliferation occurring in the cortical tissues overlying the initiating primorida, accompanied by complex auxin-cytokinin dynamics, suggesting wide-spread initiation of meristematic activity. High-resolution cross-species comparisons can allow us to derive evolutionarily preserved mechanisms for meristem initiation.

[1] Efroni I et al. (2016) Cell 165:1721-1733.

[2] Birnbaum K. (2016) Current opinion in plant biology 34:61-67.

[T1.3] The VISUAL system visualizes key regulators of early vascular development

Yuki Kondo, Alif Meem Nurani, Masato Saito and **Hiroo Fukuda**

Department of Biological Sciences, University of Tokyo, 113-0033, Japan

Xylem occupies the largest amount of territorial biomass. Vascular stem cells called (pro)cambium continues to give rise to xylem cells as well as phloem cells. We have revealed that tracheary element differentiation inhibitory factor (TDIF), a twelve-amino acid peptide, regulates vascular stem cell fate through its receptor, TDR/PXY and downstream factors, glycogen synthase kinase 3 proteins (GSK3s) (1, 2, 3). Using an inhibitor of plant GSK3s, bikinin, we succeeded in establishing a novel vascular differentiation culture system, the Vascular cell Induction culture System Using Arabidopsis Leaves (VISUAL) system, in which mesophyll cells differentiate into tracheary elements and sieve elements via procambial cells (4, 5). Using the VISUAL system, we have identified various factors that regulate distinct stages of vascular development. In this paper, we will report three key regulators, DELLA, the BES1 family and NAC020, which act at procambial cell differentiation, differentiation from procambial cells into both xylem and phloem cells, and early phloem differentiation, respectively.

[1] Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N., and Fukuda, H. (2006) Science 313: 842–845.

[2] Hirakawa Y., Shinohara, H., Kondo, Y., Inoue, A., Nakanomyo, I., Ogawa, M., Sawa, S., Ohashi-Ito, K., Matsubayashi, Y., and Fukuda, H. (2008) Proc. Natl. Acad. Sci. USA 105: 15208-15213.

[3] Kondo, Y., Ito, T., Nakagami, H, Hirakawa, Y., Saito, M., Tamaki, T., Shirasu, K., and Fukuda, H. (2014) Nature Com. 5: 4505.

[4] Kondo, Y., Fujita, T., Sugiyama, M., and Fukuda, H. (2015) Mol. Plant 8: 612–621.

[5] Kondo, Y., Nurani, A.M., Saito, C., Ichihashi, Y., Saito, M., Yamazaki, K., Mitsuda, N., Ohme-Takagi, M., and Fukuda, H. (2016) Plant Cell 28: 1250-1262.

[T2.1] The evolution of strigolactone and karrikin receptors in plants with specialized germination strategies

Caitlin E. Conn, Claudia Sepulveda, and **David C. Nelson**

Department of Botany and Plant Sciences, University of California, Riverside, USA

Obligate parasites in the Orobanchaceae have evolved the ability to remain dormant in the soil seed bank until chemical cues exuded from a nearby host root are detected, activating seed germination. This adaptation increases the likelihood of successful attachment to a host. Strigolactones are the most well-known of the host-derived germination stimulants that have so far been identified, but how they are recognized by parasitic plants has been a longstanding mystery. Over the past decade, the core strigolactone signaling mechanism has been defined in model plant systems. In parallel, we have studied the mechanism of karrikin perception. Karrikins are chemicals found in smoke that activate germination of many species after a fire. Karrikins share a butenolide moiety with strigolactones, but are otherwise chemically distinct. Strigolactones and karrikins also have unique effects on plant growth; notably, karrikins but not strigolactones promote *Arabidopsis thaliana* germination, and karrikins do not activate parasite germination (1).

Remarkably, strigolactone and karrikin signaling pathways are composed of paralogous receptors and downstream effectors, and share a common requirement for an F-box protein, indicating a shared evolutionary origin (1). We investigated how strigolactone perception evolved in parasitic Orobanchaceae. We found evolutionary, structural, and functional evidence that in parasites, *KAI2/HTL* - which regulates seed germination and mediates karrikin responses in *Arabidopsis* - had undergone extensive gene duplication and neofunctionalization that resulted a switch to strigolactone perception (2). We have analyzed the evolutionary history of KAI2 diversification in land plants and predict that a similar pattern of neofunctionalization occurred in the basal land plant *Physcomitrella patens* (3,4). We are now investigating how KAI2 become highly sensitive to karrikins, with the aim of determining whether a common set of KAI2 residues have evolved convergently in fire followers.

[1] Waters MT, Gutjahr C, Bennett T, Nelson DC. (2017) Annu Rev Plant Biol 68:291–322.

[2] Conn CE, et al. (2015) Science. 349:540–543.

[3] Lopez-Obando M, et al. (2016) Planta. 243:1441–1453.

[4] Bythell-Douglas R, et al. (2017) BMC Biol. 15:52.

[T2.2] Cross kingdom lipid transfer from plants to arbuscular mycorrhiza fungi

Caroline Gutjahr

Faculty of Biology, Genetics, University of Munich (LMU), Biocenter Martinsried, Germany and Plant Genetics; Technical University of Munich, Freising-Weihenstephan, Germany

Arbuscular mycorrhiza (AM) symbioses contribute to global carbon cycles as plant hosts divert up to 20% of photosynthate to the obligate biotrophic fungi. Previous experiments suggested that carbon is transferred to AM fungi exclusively in the form of carbohydrates. However, recently sequenced AM fungal genomes lack genes encoding subunits of cytosolic fatty acid (FA) synthase, suggesting that AM fungi may not only depend on sugar but also on FA supply from the host. We identified two *Lotus japonicus* mutants defective in AM-specifically expressed lipid biosynthesis genes. These mutants perturb arbuscule branching and the formation of vesicles. They also lack emblematic fungal 16:1ω5 FAs, suggesting that the fungus may lack sufficient amounts of FAs for desaturation. We used ^{13}C-glucose labelling followed by 16:0 and 16:1ω5 FA isotopologue profiling in roots and in extraradical fungal mycelia to address whether lipids are transferred from host plants to AM fungi. ^{13}C patterns of fungal FAs recapitulated those of two different wild-type plant hosts, indicating cross-kingdom transfer of a 16:0 FA containing lipid from plants to fungi. Transfer of labelled FAs was strongly reduced for the AM-specific lipid-biosynthesis mutants (1). This indicates that growth and development of beneficial AM fungi is not only fueled by sugars but in addition by energy-rich lipids from plant hosts.

[1]Keymer A, Pimprikar P, Wewer V, Huber C, Brands M, Bucerius SL, Delaux PM, Klingl V, von Roepenack-Lahaye E, Wang TL, Eisereich W, Dörmann P, Parniske M, Gutjahr C (2017) eLife. 6. pii: e29107.

[T2.3] Temperature sensing and signal integration in plant development

Jaehoon Jung, Daphne Ezer, Martin Balcerowicz, Xuelei Lai, Betty Chung, Meixuezi Tong, Hui Lan, David Schoepfer, and **Philip A. Wigge**

The Sainsbury Laboratory, Cambridge University, CB2 1LR, UK

Temperature has a major role in plant growth and development, and plants are sensitive to small differences in temperature[1]. For example, the phenology and distribution of wild plants has already altered in response to climate change. Despite the well-described responses of plants to higher temperature, the mechanisms of temperature perception and signal integration are not clear.

To help understand how plants sense and respond to temperature, we have used forward genetic screens with *HSP70:LUC* reporters and exploited natural variation in thermal responsiveness. From these studies, two major classes of warm temperature responses can be described, a heat stress response pathway, mediated by HSF class transcription factors and involving H2A.Z-nucleosomes[2], and a developmental and growth response, controlled by phytochrome and circadian clock signaling. Using natural variation, we have found key roles for the circadian clock (via the evening complex genes *EARLY FLOWERING3* and *LUX ARRYTHMO*) as well as *PHYTOCHROMES*[3]. We are undertaking biochemical and genetic approaches to understand how these components may be sensing temperature and if this is a direct mechanism. We and others have described evidence that phytochromes serve as thermosensors through their rate of dark reversion being proportional to temperature[4,5], and we are investigating other molecular systems that can also provide temperature information to the cell.

[1]Wigge, P. A. Ambient temperature signalling in plants. Current Opinion in Plant Biology 16, 661–666 (2013).

[2]Kumar, S. V. & Wigge, P. a. H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell 140, 136–47 (2010).

[3]Box, M. S. et al. ELF3 controls thermoresponsive growth in Arabidopsis. Curr. Biol. 25, 194–9 (2015).

[4]Legris, M. et al. Phytochrome B integrates light and temperature signals in Arabidopsis. Science (80-.). 354, (2016).

[5]Jung, J.-H. et al. Phytochromes function as thermosensors in Arabidopsis. Science (80-.). 354, (2016).

[T2.4] Control of VRN2 stability by the N-end rule pathway links oxygen sensing to PRC2 function in flowering plants

Daniel J. Gibbs

School of Biosciences, University of Birmingham, Birmingham, UK

The polycomb repressive complex 2 (PRC2) controls epigenetic gene repression in eukaryotes. Although many functions for PRC2 are known, our knowledge of post-translational mechanisms operating on this complex is limited. In the flowering plant *Arabidopsis thaliana*, the PRC2 subunit VERNALIZATION2 (VRN2) accumulates in cold temperatures, where it regulates the epigenetic memory of winter to ensure that flowering occurs in spring, a process termed vernalization (1,2). A mechanism for this environmentally-determined stabilisation has remained elusive. Here we show that VRN2 is a substrate of the N-end rule pathway, a conserved division of the ubiquitin proteasome system that targets proteins for degradation based on their N-terminal residue (3). This pathway was previously shown to coordinate oxygen and nitric oxide sensing in plants, through controlling the stability of ERFVII transcription factors (4, 5). Similarly to ERFVIIs, degradation of VRN2 is dependent on its Met-Cys-initiating N-terminus (N-degron), which links its stability to oxygen availability, and restricts its accumulation under normoxic (i.e. oxygen-replete) and non-vernalizing (i.e. warmer) conditions. We found that vernalization induces a hypoxia-like state that facilitates VRN2 stabilisation and function, providing a mechanism for its cold-responsive accumulation and activity. Furthermore, we have identified a novel function for VRN2 as a positive regulator of hypoxia and waterlogging survival. Interestingly, although PRC2s are widely conserved in eukaryotes, VRN2 orthologues in animals and early-evolving plants lack Met-Cys N-degrons. Our phylogenetic and biochemical studies suggest that VRN2 was coupled to the N-end rule in angiosperms following gene-duplication and N-terminal truncation of an ancient orthologue that contains a latent internal N-degron. We propose that this evolutionary co-option to the N-end rule pathway facilitated neo-functionalisation of PRC2 in the flowering plant lineage.

[1]Wood CC et al (2006) PNAS. 103(39):14631-6

[2]Gendall AR, Levy YY, Wilson A, Dean C (2001) Cell. 107(4):525-35

[3]Gibbs DJ, Bacardit J, Bachmair A, Holdsworth MJ (2014) Trends in Cell Biology. 24(10):603-11

[4]Gibbs DJ *et al.* (2011) Nature. 479(7373):415-8

[5]Gibbs DJ *et al.* (2014) Molecular Cell. 53(3):369-79

[T2.5] Regulation of ubiquitin-dependent intracellular membrane transport processes in plants

Marie-Kristin Nagel[1], Karin Vogel[1], Kamila Kalinowska[2,3], Cornelia Kolb[2] and **Erika Isono[1, 2]**

[1] Physiology and Biochemistry, University of Konstanz, Konstanz, Germany
[2] Plant Systems Biology, Technische Universität München, Freising-Weihenstephan, Germany
[3] Current affiliation: Cell Biology and Plant Biochemistry, University of Regensburg, Regensburg, Germany

Securing yield and guaranteeing yield quality requires the molecular understanding of mechanisms that regulate various processes throughout the plant life cycle. To recognize environmental changes, plasma membrane localized receptors and transporters play essential roles in that they translate extracellular stimuli to intracellular signaling pathways. Many of these integral membrane proteins are regulated both transcriptionally and post-transcriptionally, as their abundance at the cell membrane is key to many responses that are crucial for yield stability. One of the mechanisms that allow a tight regulation of these key regulators is the selective endocytic degradation of these factors that occurs in a ubiquitin-dependent manner. The ubiquitination status and thus also the stability of the proteins are regulated by the activity of both ubiquitylating- and deubiquitylating enzymes (DUBs). AMSH (ASSOCIATED MOLECULES WITH THE SH3-DOMAIN OF STAM) proteins are DUBs that are essential for endocytic protein degradation. We found that AMSH proteins are necessary for plant growth and development, organelle biogenesis, autophagic nutrient recycling and pathogen response in the model plant *Arabidopsis thaliana*[1,2]. Together with their interacting proteins such as subunits of endosomal sorting complex required for transport (ESCRT), ALIX (ALG-2-INTERACTING PROTEIN X) and SH3P2 (SH3-DOMAIN CONTAINING PROTEIN 2), Arabidopsis AMSH proteins act as an important component of the endosomal degradation pathway[3, 4]. In an ongoing study, we aim to elucidate the molecular mechanisms by which these factors regulate cellular trafficking pathways and diverse physiological processes in plants.

[1] Katsiarimpa and Kalinowska et al. (2013) Plant Cell. 25(6):2236-52
[2] Kolb et al. (2015) Plant Physiology, 167(4):1361-73
[3] Kalinowska et al. (2015) Proc Natl Acad Sci U S A, 2015 Oct 6;112(40):E5543-51
[4] Nagel et al. (2017) Proc Natl Acad Sci U S A, 2017 Aug 22;114(34):E7197-E7204

[T3.1] Unraveling the protein composition of plasmodesmata of Arabidopsis leaves

Max E. Kraner, Katrin Link, Müller C, **Uwe Sonnewald**

Division of Biochemistry, Department of Biology, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

Plasmodesmata (PD) are specialized tubular structures bridging plasma membranes and the cell wall of neighboring plant cells. They allow bidirectional exchange of nutrients, RNA and signal molecules between connected cells and build the basis for a supracellular network. As checkpoints for cell-to-cell communication, PD play a central role in coordinating plant growth and development but are also important for systemic viral spread. Despite these important functions, the molecular composition of PD is not well understood. To uncover components relevant for PD development we made use of the 17 kDa movement protein (MP17) of the *Potato leafroll virus* (PLRV). The protein is required for cell-to-cell movement of the virus and localizes to branched PD in source tissues. By forward genetic screening for *Arabidopsis thaliana* EMS-mutants with altered PD binding of MP17, the Choline transporter-like 1 (CHER1) protein was identified as being essential for normal PD development. Callose staining of the *cher1* mutants suggested a significant reduction in PD number of source leaves. Transmission electron microscopy of the shoot apical meristem as well as of developing and fully developed leaves confirmed the reduced PD number. The most severe reduction was found in fully developed leaves, with up to 10-times less PD compared to control plants (1). To identify PD-associated proteins, this mutant was subjected to a comparative proteomic analysis. Using a high resolution Orbitrap Fusion mass spectrometer, more than 5000 proteins could be identified in cell wall fractions of wild-type and cher1 mutant leaves. These were taken as basis for quantitative comparison. Stringent filtering resulted in a short list of 61 potential PD candidates. Amongst those, previously described PD-associated proteins were enriched (2). GFP or RFP fusion experiments confirmed PD localization of selected candidates. Functional analysis of these candidates will allow to unravel the molecular composition of PD and potential provides new insights into the regulation of PD development and function.

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[2] Kraner ME, Müller C, Sonnewald U. (2017) Plant J. doi: 10.1111/tpj.13702 epub ahead of print.

[T3.2] The atypical Arabidopsis GPI-anchored β-1,3 glucanase ZERZAUST controls floral organ development mediated by the atypical receptor-like kinase STRUBBELIG

Prasad Vaddepalli[1], Lynette Fulton[1], Jennifer Wieland[1], Katrin Wassmer[1], Milena Schaeffer[2], Stefanie Ranf[2], and **Kay Schneitz[1]**

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Orchestration of cell division and anisotropic growth patterns in plant organogenesis requires intercellular communication and the accommodation of cell wall dynamics. The underlying signaling mechanisms are poorly understood. In Arabidopsis floral morphogenesis depends on signal transduction mediated by the receptor-like kinase (RLK) STRUBBELIG (SUB) (1). SUB is unusual as it represents an atypical or "dead" RLK. How signal transduction is mediated by atypical RLKs is largely unexplored. Our recent data indicated that SUB physically interacts with the multiple C2 domain protein QUIRKY (QKY) at plasmodesmata, channels interconnecting most plant cells (2). Moreover, non-cell-autonomy of SUB and QKY suggested that they function by regulating an unknown mobile signal (SMS).

Previous genetic work identified ZERZAUST (ZET) as a further core component of SUB signaling (3). Here we provide a molecular and functional characterization of ZET (4). The results provide first evidence connecting SUB-mediated signal transduction to cell wall biology. We show that ZET is a mobile cell wall protein. ZET is a putative β-1,3 glucanase suggested to degrade the cell wall polymer callose. However, several lines of evidence indicate that ZET is catalytically inactive. This implies that ZET controls cell wall chemistry in a regulatory manner. Indeed, our data reveal that SUB, QKY and ZET affect cell wall structure. We propose that SUB, QKY and ZET promote tissue morphogenesis through connecting cell wall remodeling, receptor-like kinase signaling and plasmodesmata-associated inter-cellular communication.

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[T3.3] A structural journey among floral regulators

François Parcy

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In Arabidopsis like in many other plants, the development of flowers starts on the flanks of the shoot apical meristem. The position of the floral meristem is determined by the phytohormone auxin and the Auxin Response Factor Monopteros/ARF5 protein. Soon afterwards, the LEAFY transcription factor contributes to the emergence and the floral fate of the newly formed flower meristem. I will present how the combination of molecular genetics, structural biology, genomics and modelling allows to better understand the basic properties of these transcription factors and their function during flower development.

[T3.4] Genetic regulation of spike form and grain number in temperate cereals

Thorsten Schnurbusch, Naser Poursarebani, Helmy Youssef, Ravi Koppolu, Shun Sakuma, Twan Rutten

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; Independent HEISENBERG-Research Group Plant Architecture

My lab is interested in the molecular-genetic elucidation of early inflorescence development in small grain cereals, specifically wheat and barley. Functional knowledge of genes, which regulate key developmental traits such as inflorescence branching, spikelet initiation or abortion, rachis internode length, or total number of rachis internodes is almost completely lacking in these important cereal crops. To this end, we are utilizing natural spike variants from wheat and induced spike mutants from barley to clarify the genetic make-up of genes underlying developmental phenotypes for reduced and increased grain number per spike (1). Here I will report our latest results relating to genes which alter spike shape and their effects on spikelet fertility. Moreover, I will share with you our latest results on a transcriptional regulator that is specifically involved in floral organ patterning and phase duration by maintaining hormonal homeostasis and gradients during normal spike development; but similarly influenced plant stature traits (2). Collectively, I will provide new insights into the genetic basis of spike architecture in Triticeae thereby potentially disclosing new targets for boosting yield potential.

[1] Poursarebani et al. (2015) Genetics 201: 155-165

[2] Youssef et al. (2017) Nat. Genet. 49: 157-161

[T4.1] Exporters – the missing link in long-distance transport of amino acids

Julia Karmann, Anna-Theresa Dandekar, Benedikt Müller, Astrid Fastner and **Ulrich Z. Hammes**

Plant Systems Biology, Technische Universität München, Freising-Weihenstephan; Cell Biology and Plant Biochemistry, Biochemie-Zentrum Regensburg, Universität Regensburg, Germany

Amino acids represent the main transport form of reduced nitrogen in plants. Following uptake or assimilation, amino acids are transported within the plant in the both tissues of the vasculature, the phloem and the xylem. In order to be loaded into the vascular tissues amino acids must at some point be secreted from the cells into the apoplast. Also, to unload the vasculature in tissues that import amino acids, transporters are required to catalyze the release.

We have identified a family of amino acid facilitators that belong to a plant-specific family of transporters, called UmamiTs, that play a crucial role in these processes. We show that four UmamiTs localize to the plasma membrane of the vascular parenchyma suggesting a role in the transfer of amino acids between the phloem and the xylem and consequently a role in loading processes in source tissues and unloading processes in sink tissues. This is substantiated by the observation that *umamit* single mutants produce smaller organs, including seeds. Furthermore, the reproductive success of vasculature-dependent parasitic nematodes correlates directly with *UmamiT* gene dosage. Higher complexity mutants are lethal or show severe developmental defects which are due to reduced meristem activity. Our data suggest that UmamiTs are the long-sought exporters required for proper long-distance transport of amino acids and key players in nitrogen efficiency.

[T4.2] Building and bypassing plant polyspermy barriers

Thomas Nakel, Dawit Tekleyohans, Golo Fuchert, Dieu Vo, and **Rita Groß-Hardt**

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The ultimate goal for the survival of all species on earth is to reproduce. Flowering plants are internally fertilized and utilize pollen tubes for sperm transport. While initially an armada of pollen tubes sets out to target the ovules, an individual ovule is typically only conquered by a single pollen tube. Supernumerary pollen tube attraction is prevented by a pollen tube block, which is mounted after fertilization (1-3). Key to the establishment of the pollen tube block is the degeneration of pollen tube attracting synergid cells, a process that requires Polycomb Repressive Complex 2 and an ethylene response cascade (3, 4). Here, we provide insights into some of the mechanisms underlying the establishment of the pollen tube block and discuss the developmental consequences associated with defective polyspermy barriers.

[1] Kasahara R, Hamamura Y, Sakakibara Y, Twell D, Higashiyama T (2012) Curr. Biol. 22, 1084-89.

[2] Beale KM, Leydon A, Johson MA (2012) Curr. Biol. 22, 1090-94.

[3] Maruyama D, Hamamura Y, Takeuchi H, Susaki D, Nishimaki M, Kurihara D, Kasahara RD, Higashiyama T (2013) Dev. Cell 25, 317-23.

[4] Völz R, Heydlauff J, Ripper D, von Lyncker L, Groß-Hardt R (2013) Dev. Cell 25, 310-16.

[T4.3] Molecular and cellular events during flowering plant gamete interactions

Maria Englhart, Philipp Cyprys, Thomas Hackenberg, Maria Flores-Tornero and **Stefanie Sprunck**

Cell Biology and Plant Biochemistry, Biochemie-Zentrum Regensburg, Universität Regensburg, Germany

Fertilization describes the successful fusion of two haploid gametes of opposite sex to initiate the development of a new diploid organism. Flowering plants have evolved the unique mechanism of double fertilization in which two female reproductive cells (egg cell and central cell) get fertilized by two male gametes (sperm cells) (1). The two fertilization products, the embryo and the endosperm, constitute the edible parts of many seeds and grains and provide most of our plant-based food and livestock feed. Given the agronomic importance, the molecular understanding of double fertilization is one of the preconditions to improve seed set and yield stability.

The molecular processes that induce one flowering plant sperm cell to attach to and to fuse with the egg cell, while the second sperm cell almost simultaneously fuses with the central cell, remain to be understood. Nevertheless, it is evident that gamete recognition, tight adhesion and fusion is mediated by molecules acting on the cell surfaces. To identify cell surface proteins involved in gamete interactions we performed transcriptomics and membrane proteomics with isolated male and female gametes of different flowering plant species such as wheat, maize, and Arabidopsis. Results of these approaches and the analyses of candidate membrane proteins will be presented, such as the family of small cysteine-rich EC1 (EGG CELL 1) proteins, which are secreted by the Arabidopsis egg upon sperm cell delivery to achieve rapid sperm activation and gamete fusion (2). The transcriptome data also enabled us to generate marker lines for the visualization of egg-sperm membrane interactions *in vivo* and to monitor the cytosolic Ca^{2+} dynamics in the female gametes of Arabidopsis using live-cell imaging. These experiments provide strong evidence for a role of Ca^{2+} signaling during double fertilization as each female gamete displays a characteristic calcium signature differing by timing and behavior from cytoplasmic Ca^{2+} waves reported in animals (3).

[1] Dresselhaus T, Sprunck S, Wessel GM (2017) Current Biol. 26, R125–R139.
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[3] Denninger P, Bleckmann A, Lausser A, Vogler F, Ott T, Ehrhardt DW, Frommer WB, Sprunck S, Dresselhaus T and Grossmann G (2014) Nature Comm. 5, 4645.

[T5.1] Improvement of wheat in the era of New Breeding Methods

Klaus Schmidt

KWS Saat SE; Einbeck, Germany

In the past genomic modifications in wheat were difficult to achieve due to inefficient technologies, like transformation technology. In addition, the genome of wheat is very complex, making alternative approaches for modification like TILLING very challenging or sometimes even impossible.

In the last years, a significant improvement in the development of a highly efficient transformation technology in wheat has been achieved. Together with the development of efficient designer nucleases like TALENs or CRISPR-nucleases, targeted modifications in the complex wheat genome are now possible and can be used to create new or improve traits in this important crop. Although the major technical bottlenecks for the creation of improved wheat were eliminated, the commercialization of such wheat varieties is still not possible, due to the transgenic status of those plants which leads to non-acceptance of consumers and the value chain in Europe.

So called New Breeding Methods, which are available now in the tool box of plant breeders, provide a unique opportunity going forward also for wheat breeding. Breeding goals can be reached faster and more precisely, and genetic variation can be broadened for greater diversity of varieties. By using New Breeding Methods, there are applications which do result in non-transgenic products. Nevertheless, plant breeders are far away from having legal certainty in using these new methods as the regulatory evaluation is still ongoing, at least in Europe.

In my talk, I would like to give an overview about the development of an efficient wheat modification system. I would also like to draw your attention to the today's regulatory uncertainties in Europe when applying New Breeding Methods.

[T5.2] Dissecting C4 photosynthetic pathways in the grasses; from comparative genomics to functional genomics

Tom Brutnell

Danforth Center, St. Louis, USA

The grasses represent some of the world's most important feed, food and bioenergy crops and have been the focus of breeding efforts for over 10,000 years. Recent advances in genome sequencing, genome editing and transformation promise to accelerate the rate of gain in crop productivity in the decades ahead, but this will require a more sophisticated analysis and mining of massive genomic datasets and more streamlined testing of improved and novel genetic circuits. We are developing new algorithms and techniques to mine genome sequence and transcriptomic datasets to identify novel components of the C4 photosynthetic pathway with the goal of manipulating these components to increase photosynthetic efficiencies and ultimately yield in C4 grasses that include maize, sorghum and the millets. We are also developing tools for the model grass *Setaria viridis* to enable the rapid dissection of genes and pathways as well as a platform for testing synthetic circuits. Resources that have been developed for *S. viridis* include a large NMU-mutagenized population, the development of high efficiency transient and stable transformation and the deployment of CRISPR-Cas9 genome editing technologies. I will present data on the discovery and manipulation of both biochemical and anatomical features required for C4 photosynthesis and discuss our ongoing work to manipulate these pathways to increase crop yields.

[T5.3] Genetic control of spikelet development in barley

Maria von Korff

Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding Research, Köln; Institute for Plant Genetics, Heinrich-Heine-Universität Düsseldorf; Cluster of Excellence in Plant Sciences, Heinrich-Heine-Universität Düsseldorf, Germany.

Variation in the number of seeds per spike has a large impact on grain yield in barley. The number of seeds per spike is affected by early developmental processes that determine the number of initiated spikelet primordia, by further spikelet development and floret abortion. We found that the *Photoperiod Response* gene *Ppd-H1* affects floral development and floret survival under long days and high ambient temperatures. Global transcriptome analysis in developing shoot apices revealed candidate genes that correlated with floral development. Among these candidate transcripts we detected known regulators of lateral spikelet development and thus spike row-type. In addition, we identified and characterized the gene underlying the intermediate row-type mutant *six-rowed spike 3* (*vrs3*) as a putative histone Lysine demethylase based on RNA-sequencing in allelic *vrs3* mutants. The transcriptome data suggested that VRS3 acts as a transcriptional activator of the row-type genes *VRS1* (*Hv.Hox 1*) and *INTERMEDIUM-C* (*INT-C; Hv.TEOSINTE BRANCHED1*). Comparative transcriptome analysis of the row-type mutants *vrs3*, *vrs4* (*Hv.RAMOSA2*), and int-c confirmed that all three genes act as transcriptional activators of *VRS1* and quantitative variation in the expression levels of *VRS1* in these mutants correlated with differences in the number of fertile lateral spikelets. The identification of genes and pathways affecting seed number in small grain cereals is an important step towards improving overall grain yield.

[T6.1] Maintenance of Polycomb gene silencing depends on H3 variants in plants

Danhua Jiang, Haifeng Wang, and **Frédéric Berger**

Gregor Mendel Institute, Austrian Academy of Sciences, Vienna Biocenter, Vienna, Austria

Propagation of patterns of gene expression through the cell cycle requires prompt restoration of epigenetic marks after the twofold dilution caused by DNA replication. We show that plants evolved a mechanism for efficient K27 trimethylation on H3.1, which is essential for inheritance of the silencing memory from mother to daughter cells. The transcriptional repressive mark histone H3 lysine 27 trimethylation (H3K27me3) is restored in replicating plant cells through DNA replication-coupled modification of histone variant H3.1.[1] In non dividing cells, the maintenance of H3K27me3 is relayed by ATRX, which controls H3.3 deposition. We illustrate how H3 variants collaborate to assist H3K27me3 mediated silencing during the developmental transition to flowering.

[1]Jiang D and Berger F. (2017) Science. *DOI: 10.1126/science.aan4965*

[T6.2] Genome engineering in plants: Past, Present, Future

Holger Puchta

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Sequence-specific nucleases can be used to induce double-strand breaks (DSBs) in plant genomes. In the past we could show that thus gene targeting (GT) by homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Moreover, by inducing several DSBs, sequences can be deleted out of the genome or chromosome arms exchanged. In the last years CRISPR/Cas became the major tool for targeted mutagenesis. We were able to demonstrate *Streptococcus pyogenes* (Spy)Cas9 nuclease induced, NHEJ mediated, heritable targeted mutagenesis in *Arabidopsis thaliana as well as homology dependent in planta GT*. Off-target effects might be avoided using two sgRNAs and a Cas9 protein that was transformed from a nuclease to a nickase, to induce adjacent single strand breaks (SSBs) in opposite strands. This “paired nickase” strategy results in DSBs with 40 instead of 20 basepair specificity. Interestingly; sequence duplications are a prominent outcome of this approach, hinting to the possibility that the repair of adjacent SSBs is a major cause of sequence duplications during genome evolution of plants. Recently we applied the Cas9 orthologues from *Streptococcus thermophilus* (Sth1Cas9) and *Staphylococcus aureus* (SauCas9) for NHEJ-mediated targeted mutagenesis in *A. thaliana* with efficiencies at least comparable to those of SpyCas9. We were also able to show that the SauCas9 and SpyCas9 proteins only work in the presence of their species-specific single guide (sg) RNAs and show no inter-species interference. Thus, the Cas9 proteins of *S. pyogenes* and *S. aureus* should be appropriate for simultaneously addressing different sequence motifs with different enzyme activities in the same plant cell. Using nuclease dead fluorescence fusion of both proteins we together with the group of Andreas Houben from the IPK Gatersleben were able to show that sequence specific visualization of repeated sequences in the Arabidopsis genome The simultaneous use of different Cas9 orthologues will offer the opportunity to detect or control genetic information of plant cells on more complex levels than before and will lay the basis for future synthetic approaches in plant biology.

[T6.3] Natural variation for growth response to the environment in Arabidopsis

Olivier Loudet

Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000 Versailles, France

Following a long history of quantitative genetics in crop plants, it is now quite popular as well to use naturally-occurring variation contained in *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map QTLs and try and resolve them at the gene level. Apart from being able to exploit –in multiple genetic backgrounds– allelic variation that cannot be easily retrieved from classical mutagenesis, the success of the QTL studies has often been because of the use of quantitative phenotyping, as opposed to the qualitative scales often used in typical mutant screens. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the abiotic environment (especially drought stress). We are using a combination of our unique high-troughput phenotyping robot (the Phenoscope), RNA-seq, fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale thanks to the Phenoscope should allow to resolve enough quantitative loci to start drawing a more general picture as to how and where in the pathways adaptation is shaping natural variation. I will present recent results obtained when trying to decipher the genetic architecture of dynamic growth response to the environment, to illustrate our strategies and research.

[T6.4] Deciphering the nitrogen-fixing root nodule symbioses with comparative phylogenomics

Maximilian Griesmann[1,2], Yue Chang[3], Xin Liu[3], Manuel Spannagl[2], Georg Haberer[2], Pierre-Marc Delaux[4], Shifeng Cheng[3], Klaus F. X. Mayer[2], Martin Parniske[1]

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Of all the essential plant nutrients, nitrogen is required in the largest quantity and is frequently the limiting factor in crop productivity. Although plants are surrounded by atmospheric nitrogen, they are unable to use it. Some flowering plant lineages escaped the requirement for fixed nitrogen in the soil by forming the root nodule symbioses (RNS) with nitrogen-fixing bacteria. This capability is restricted to four closely related orders: Fabales, Fagales, Cucurbitales and Rosales. As a result of this relationship, there is a hypothesis that RNS evolved many times, independently, from a predisposition event that occurred before the divergence of these orders (1). However, the molecular mechanisms that allowed RNS to evolve remain largely unknown. Through a global collaborative effort, we have sequenced the genomes and transcriptomes of 13 species that together cover the breadth of symbiotic states in the Fabales, Fagales, Cucurbitales and Rosales orders. I will present the key findings from our comparative phylogenomics analysis of this dataset.

[1]Soltis D, Soltis P, Morgan D, Swensen S, Mullin B, Dowd J, Martin P. (1995) PNAS 92(7):2647-2651.

[T7.1] Core effectors in smut fungi: an amazing treasure box

Lay-Sun Ma, Nicole Ludwig, Liang Liang, Gabriel Schweizer, Timo Glatter, Stefanie Reissmann and **Regine Kahmann**

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The fungus *U. maydis* causes smut disease in maize. *U. maydis* is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection, *U. maydis* needs to suppress plant defense responses and manipulate host physiology for its own benefit. To accomplish this, *U. maydis* secretes a cocktail of several hundred effector proteins. The majority of these proteins lack known protein domains and their function remains to be uncovered. Based on a comparative analysis of six smut genomes we have identified a set of core effectors which are present in all six species. A systematic deletion of the most highly expressed effector genes in this class resulted in the discovery of mutants with strong virulence phenotypes. One of these is the repetitive effector Rsp3. We present evidence that this effector is bound to the fungal surface and inhibits the activity of an antifungal maize protein. Interestingly, another four individual, unrelated core effectors from this group are absolutely essential for virulence. *stp1, stp2, stp3 and stp4 mutants* (stop after penetration) deletion strains are able to form appressoria that penetrate, but their growth is arrested in epidermal host tissue. A similar phenotype was also observed for mutants lacking the essential effector *pep1* (1). The observed growth arrest was in all cases accompanied with the elicitation of plant defense responses and plant cell death. Co-IP with individually tagged effectors followed by mass-spectroscopic analysis revealed that Stp1, Stp3, Stp4 and Pep1 form a complex. I will discuss our current efforts to localize the complex and to functionally characterize its components and discuss possible scenarios for the function of the complex.

[1] Doehlemann G, van der Linde K, Assmann D, Schwammbach D, Hof A, Mohanty A, Jackson D, Kahmann R (2009). PLoS Pathog. 2009 Feb;5(2)

[T7.2] Leaf microbial communities: structure and dynamics

Matthew T. Agler, Jonas Ruhe, Samuel Kroll, Alfredo Mari, Juliana Almario, Ariane Kemen and **Eric Kemen**

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Plant-associated microorganisms critically affect host phenotypes including growth, disease and reproductive fitness. While the ability to control plant microbial communities is central to ensuring food security by advances in resource-efficient crop production, increased yield and protection, our basic understanding of how microbial communities assemble and how they persist on plants and become robust to perturbation is still very limited.

We addressed this fundamental knowledge gap by simultaneously studying wild and planted *Arabidopsis thaliana* populations in the field and used reconstitution biology in gnotobiotic systems to dissect mechanisms.

Our results indicate that abiotic factors, host genotype and time together have strong effects on plant colonization patterns of bacteria, fungi, oomycetes and other protists (1). Only a minority of all microbes we identify, however, are strongly interconnected with other microbes and have a severe effect on community structure development. We therefore call those ‘microbial hubs’. Those ‘hubs’ via host-microbe and microbe-microbe interactions transmit host genotypic signatures to the microbial community structure. In depth analyses on ‘hub’ microbes revealed strong effects on endophytic colonization for specific taxonomic groups from phyllosphere communities that otherwise vary between plants.

The identification of microbial ‘hubs’ and their importance in phyllosphere microbiome structuring over time has crucial implications for plant-pathogen and microbe-microbe research and opens new entry points for ecosystem management and future targeted biocontrol (2).

[1] Agler MT, Ruhe J, Kroll S, et al. (2016) PLoS Biol 14:e1002352

[2] Kroll S, Agler MT, Kemen E (2017) Curr Opin Plant Biol 36:71-78

[T7.3] Dynamic patterning of cell surface receptors and partners

Thomas F. Stratil[1], Pengbo Liang[1,2], Corinna Buschle[1], Nikolaj Abel[1,2], Casandra Hernández-Reyes[2], Claudia Popp[1], Macarena Marín[1], Jessica Folgmann[1] and **Thomas Ott**[1,2]

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Considering the dense packing of proteins at the plasma membrane and the plethora of environmental stimuli that tissues need to perceive and integrate simultaneously, cells evolved membrane-based substructures called membrane nanodomains (NDs) (1). These diverse compartments may serve as central hubs for the specific assembly of signalling complexes including receptors. Even though many individual proteins have been described to laterally segregate into NDs at the cell surface (2), evidence presented recently proofed for the first time that functionally divergent receptors localize to different NDs (3). Here, we will report on molecular mechanisms that regulate lateral mobility of membrane resident receptors and prevent ligand-induced receptor endocytosis during host cell infection. Latter analysis together with the discovery of key molecular building blocks that are required and sufficient to maintain an infection-related ND in vivo enabled us to propose a sequence of events during ND assembly in living cells.

[1] Ott T. (2017) Curr Op Plant Biol. 40:82-88.

[2] Jarsch IK, Konrad SSA, Stratil TF, Urbanus SL, Szymanski W, Braun P, Braun KH, Ott T (2014) Plant Cell 26:1698-1711.

[3] Bücherl CA, Jarsch IK, Schudoma C, Segonzac C, Mbengue M, Robatzek S, MacLean D, Ott T and Zipfel C (2017) eLIFE 6:e25114

[T7.4] A novel component of the CCaMK/CYCLOPS complex regulates root nodule symbiosis

Martin Parniske

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Legumes form symbiosis with phosphate-acquiring arbuscular mycorrhiza fungi and nitrogen-fixing rhizobia. Early developmental stages of both symbioses are characterized by calcium-spiking in the nucleoplasm, which is likely to be decoded by a complex formed by a calcium- and calmodulin-dependent protein kinase (CCaMK) and CYCLOPS, a DNA-binding transcriptional activator (1,2,3). This complex occupies a key hierarchical position in symbiosis signaling, because deregulated versions of either CCaMK or CYCLOPS are able to induce spontaneous symbiotic root responses in the absence of microsymbionts (1). However, several lines of evidence indicate that additional complex components, such as DELLA, are involved in tuning the activity of the complex (2). Here we provide structural insights into CYCLOPS regulation during root symbiosis. Using x-ray crystallography, part of the CYCLOPS DNA-binding domain was structurally solved. Based on the protein structure, a novel component of the CCaMK/CYCLOPS complex could be predicted. The predicted interaction was confirmed by independent protein-protein and protein-DNA interaction assays. The phenotype caused by a mutant allele of the novel gene revealed its regulatory role in symbiosis. Our data suggest that the CCaMK/CYCLOPS complex is subject to dynamic changes in composition and structure, which would be in accordance with the specific spatio-temporal requirements of transcriptional regulation during the development of a symbiotic root nodule.

[1] Singh et al. 2014 Cell Host & Microbe

[2] Pimprikar et al. 2016 Current Biology

[3] Cerri et al. 2017 New Phytologist

[T8.1] Regulation of receptor kinase-mediated immune signaling

Cyril Zipfel

The Sainsbury Laboratory, Norwich Research Park, Norwich, UK.

Cell surface receptor kinases are essential to perceive extracellular stimuli and to modulate cellular outputs during growth and development, as well as in response to environmental challenges. In plants, several pattern recognition receptors (PRRs) involved in 'non-self' innate immune perception are receptor kinases, often found as part of heteromeric kinase complexes. Appropriate immune signaling initiation, timing and amplitude must be carefully regulated to avoid excessive or unspecific activation of immune responses, which can lead to autoimmune and inflammatory diseases. The mechanisms and pathways that negatively regulate innate immunity in mammals have been extensively characterised. However, much less is known in plants, where a fine balance between immunity and growth is important for their optimal fitness. I will present recent work illustrating how PRR-mediated innate immune signaling is tightly regulated by dynamic post-translational modifications and by endogenous peptides.

[T8.2] Monoterpenes promote systemic acquired resistance within and between plants

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Plant innate immune responses include systemic aspects conferring for example enhanced resistance in the systemic parts of locally infected plants. In this case, systemic immunity is executed as a form of priming and is known as systemic acquired resistance (SAR; 1). SAR is strongly associated with salicylic acid (SA) and is believed to depend on phloem-mediated distribution of a growing number of signaling intermediates. In this work, we identified volatile signaling intermediates that are required for SAR and additionally act as airborne infochemicals propagating innate immune signaling between plants (2). Gas chromatography coupled to mass spectrometry analyses of SAR-related emissions of wild-type and non-SAR-signal-producing mutant plants associated SAR with monoterpene emissions. Headspace exposure of *Arabidopsis thaliana* to a mixture of the bicyclic monoterpenes α-pinene and β-pinene induced defense, accumulation of reactive oxygen species (ROS), and expression of SA- and SAR-related genes, including the SAR regulatory *AZELAIC ACID INDUCED1* (*AZI1*) gene and three of its paralogs (2). Additionally, the volatile emissions from SAR signal-emitting plants induced defense in neighboring plants and this was associated with the presence of α-pinene, β-pinene, and camphene in the emissions of the 'sender' plants. Recent data show that pinene-mediated propagation of immunity also acts between different species, suggesting that monoterpenoid VOCs are part of an ecologically relevant mechanism to relay signals to other plants in the nearby environment.

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[T8.3] Immune sensing of lipopolysaccharide in animals and plants: same but different

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Innate immunity, triggered after recognition of microbe-associated molecular patterns (MAMPs) by specific host receptors, is crucial for animals and plants. Cell surface components such as lipopolysaccharide (LPS), peptidoglycan and flagellin are typical MAMPs as they are vital for microbial survival and common to whole microbial classes. LPS, in particular the endotoxic lipid A moiety, of Gram-negative bacteria is sensed as MAMP in mammals through different extra- and intracellular LPS receptors. Recently, we found that the receptor-like kinase LORE (LipoOligosaccharide-specific Reduced Elicitation), which belongs to the plant-specific class of bulb-type lectin S-domain-1 kinases (SD1-RLKs), mediates sensitive perception of Pseudomonas LPS in Arabidopsis1. The lipid A moiety from Pseudomonas LPS alone is sufficient to induce LORE-dependent immune responses1.

Interestingly, LORE specifically senses Pseudomonas and Xanthomonas but not the typical enterobacterial LPS e.g. of Escherichia coli. In mammals, on the contrary, enterobacterial LPS is the most potent immune activator, whereas Pseudomonas LPS is only a weak agonist due to structural differences within the lipid A. Thus, both mammals and plants evolved to sense LPS via its lipid A moiety but, apparently, with distinct epitope specificities and through structurally unrelated receptors. Currently, we are investigating the structural features within the lipid A that determine the specificity of LORE-mediated LPS sensing in plants, and the formation and activation of the LORE receptor complex upon LPS perception.

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Poster abstracts

[P1] The contribution of the GATA transcription factors GNC and GNL in the greening of *Arabidopsis thaliana*

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The synthesis and accumulation of chlorophylls in chloroplasts lead to the greening of plants. During this process, plants become photosynthetically active and use the energy from the sun, which is mainly captured by chlorophylls, in order to assimilate CO_2 from the atmosphere and convert it to sugars. Many studies, which were conducted in the past years, focused mostly on the functional and biochemical characterization of enzymes of the chlorophyll biosynthesis pathway (1). Nevertheless, the transcriptional regulation of greening remains largely unknown. B-GATA transcription factors have a prominent role in greening (2), and the goal of this study was to elucidate their contribution to this process.

By combining, transcriptomic data with metabolic, molecular and genetic studies, we were able to show that the Arabidopsis B-GATAs GNC/GATA21 and GNL/GATA22 can control greening through five different ways: (I) Transcriptional regulation of genes encoding for enzymes in chlorophyll biosynthesis pathway (*GUN5*, *GUN4*, *CHLD*, *CHLI1/2* and *DVR*), (II) control of the expression of *GUN2*, a heme oxygenase in the phytochromobilin/phytochrome pathway, (III) direct transcriptional control of *SIGMA* factors (*SIG2* and *SIG6*), known regulators of the transcription in the chloroplasts, (IV) control of other transcription factors with pivotal role in greening (*GLK1* and *GLK2*), and finally, (V) promotion of greening through the retrograde signaling.

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[P2] LLM-Domain B-GATA transcription factors promote cell devision and stomatal development in the non-protruding cell files of *Arabidopsis thaliana* hypocotyls

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The GATA transcription factor family in plants consists of appoximately 30 members that can be subdivided into 4 families based on phylogenetic analysis, their intron-exon-structure and sequence homology of the DNA-binding GATA-domain (1). Members of the B-class of GATA transcription factors harbouring a C-terminal LLM-domain show redundancy in regulating some aspects of plant development and physiology like hypocotyl elongation and greening (2). While exploring the hypocotyl elongation phenotype, we realized that mutants of *Arabidopsis thaliana* LLM-domain B-GATA genes are defective in stomata formation in this tissue. Conversely, stomata formation and cell division is strongly promoted by overexpression of various LLM-domain B-class GATA genes, most strikingly in non-protruding cell files of the hypocotyl but also in cotyledons. The successive stages of stomata initiation, development and terminal differentiation requires the three basic helix-loop-helix (bHLH) transcription factors *SPEECHLESS* (*SPCH*), *MUTE*, and *FAMA* (4, 5, 6). While cell-to-cell signaling enforces proper distribution and density of stomata formation through the interaction of the *EPIDERMAL PATTERNING FACTOR* (*EPF*)*/EPF-LIKE* (*EPFL*) family of secreted peptides with three *ERECTA* (*ER*)-family leucine-rich repeat receptor kinases (LRR-RKs), *ER*, *ER-LIKE1* (*ERL1*) and *ERL2*, and an LRR receptor-like protein, *TOO MANY MOUTHS* (*TMM*) (7-11). Genetic analyses indicate that these B-GATAs act upstream of the stomata formation regulators *SPEECHLESS* (*SPCH*), *MUTE*, and *SCREAM/SCREAM2* and downstream or independent of the patterning regulators *TOO MANY MOUTHS* and *STOMATAL DENSITY AND DISTRIBUTION1* (3).

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[P3] Identification of gibberellin signaling components in cold stress

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The phytohormone gibberellin (GA) is implicated in multiple aspects of plant growth and development, as well as in responses to environmental stimuli such as cold-stress. Exposure of *Arabidopsis* to low temperature results in growth inhibition and transcriptional changes that lead to cold acclimation. Exposure to cold temperature also leads to a reduction of GA hormone levels and the stabilization of DELLA proteins, critical growth repressors of the GA pathway (1, 2). DELLA proteins intergrade multiple signals by interfering with the activity of a range of transcription factors through protein-protein interactions (3).

We aim to understand how GA and DELLAs regulate the cold-stress response by identifying downstream DELLA targets in this pathway. To this end, we examined the contribution of DELLA-dependent transcriptional changes in cold stress using RNA-Seq. We discovered that around 9 % of the cold-induced transcriptional changes are differentially expressed during concomitant GA application. Interestingly, application of GA affected a largely different gene set upon cold-stress than application of GA to ambient temperature-grown seedlings. The latter suggests that GA and hence the control of transcription by DELLAs depends on the different dynamics of protein-protein interactions in ambient temperature versus cold stress. To identify DELLA-regulated transcription factors that may operate during cold stress, we performed a yeast two-hybrid screen with a collection of 2000 Arabidopsis transcription factors, where we identified more than 200 DELLA interactors for the two DELLAs, RGA and GAI. These included 32 DELLA-interacting transcription factors that were also transcriptionally regulated after GA treatments in the above described transcriptomics experiment. Our focus is on the elucidation of the role of the DELLA interaction with growth-promoting transcription factors in mediating cold stress-specific GA responses. Latest results will be presented.

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[P4] A DELLA- and gibberellin-regulated co-expression framework for abiotic stress responses in plants

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Abiotic stress is a major threat for crop production. Abiotic stresses lead to increases in the abundance of the DELLA proteins, central regulators of the gibberellin (GA) pathway. DELLAs generally work as transcription factor repressors but how DELLAs control abiotic stress responses is not well understood.

In order to understand how DELLAs and GA controls abiotic stress responses, we have performed cold stress responses using time-resolved RNA-seq in Arabidopsis. The expression data generated was analyzed using weighted co-expression analysis, focusing on stress-specific GA-dependent transcriptomes, and subsequently subjected to multiple network analysis and “in-house” data-mining pipelines in order to generate groups of target genes. Similar data generated for tomato and barley was also integrated, in order to expand targeted groups for multiple species, using a family-similarity-based approach.

The current work is meant to serve as resource for GA- and DELLA-biology, and also to serve and a framework for inter-species research.

[P5] Temperature-dependent control of flowering by the gibberellin pathway and interactions between DELLA proteins and AP1/VRN1 MADS-box factors.

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The mechanism underlying the regulation of flowering time by gibberellin (GA) in the context of cold temperature is poorly understood. In *Arabidopsis*, low temperature delays growth and flowering; at the molecular level this delay can be explained by the fact that cold temperature promotes the catabolism of GA, leading to an accumulation of DELLA proteins, the major repressors of GA responses[1,2]. DELLA abundance responds to changes in temperature and the effect of DELLA accumulation can be suppressed by GA treatments. In *Arabidopsis*, barley and rice, GA has a very important role in the regulation of flowering time: GA biosynthesis mutants in these species show a strong delay in flowering time. In *Arabidopsis*, the MADS-box transcription factor APETALA1 (AP1) is well known to play a pivotal role in determining the floral meristem identity, and its expression is downstream the flowering promoting pathways[3]; more over in our laboratory it has been shown that AP1 is directly repressed by DELLAs. In barley (*Hordeum vulgare*), VRN1, the orthologue of AP1, is the master regulator of flowering time in barley[4]; in winter varieties its expression is gradually induced with exposure to cold temperature, a process known as vernalization, whereas in spring varieties the expression of VRN1 independent from a cold stimulus is the basis for their vernalization-independent flowering. We can thus hypothesize that DELLA proteins repress flowering through interactions with AP1/VRN1 and this repression is relieved, (i) by the GA-dependent DELLA degradation and, (ii) by increased expression of AP1/VRN1. We want to understand if the AP1/DELLA interaction identified in *Arabidopsis* also takes place between VRN1 and SLN1, the only DELLA protein from barley and if the flowering time control in this species, in response to GA and temperature, is dependent on this interaction. More in details, we want to understand if the delay in growth and flowering in barley at low temperature can be rescued by GA application and correlates with the effect of GA and temperature on DELLA abundance. Moreover we want to understand if and how the GA biosynthesis genes in barley are affected by cold and GA[5,6].

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[P6] Floral morphogenesis requires attenuation of a clathrin-mediated endocytotic process by the receptor kinase STRUBBELIG

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In plants, an open question remains as to what coordinates cell behavior during organogenesis, permitting organs to reach their appropriate size and shape. The Arabidopsis putative atypical leucine-rich repeat receptor kinase STRUBBELIG (SUB) regulates floral organ shape, the plane of cell division in cells of the first subepidermal cell layer of floral meristems, ovule integument morphogenesis (1-4). In order to identify additional factors of the SUB pathway regulating floral morphogenesis we searched for putative SUB-interacting proteins using the intracellular domain of SUB as bait in a yeast two-hybrid screen. We have identified the *CLATHRIN HEAVY CHAIN 2 (CHC2)* gene as a promising candidate. In vivo co-IP revealed that CHC and SUB occur in the same protein complex in plants. The combined results indicate that SUB directly interacts with CHC in planta.

We further assessed the biological function of *CHC* in *SUB*-dependent floral morphogenesis. To this end we used two independent loss-of-function alleles each of *CHC2* and the related *CHC1*. Single *chc* mutants do now show altered floral morphology. However, single *chc* knockout mutations partially suppress the silique twisting, the L2 division plane defects in floral meristems and the ovule defects of *sub-9* mutants. This result indicates that *SUB* is a negative genetic regulator of *CHC*. Taken together, our findings suggest CHC proteins to be central downstream components of SUB whose activity needs to be attenuated by SUB to allow for proper floral organ development in Arabidopsis.

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[P7] Regulatory feedback mechanism between AGC kinases controlling Arabidopsis ovule integument growth

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The spatial coordination of growth is of crucial importance for the maintenance of distinct cell layers and tissue morphogenesis. These cell layers are maintained by symmetric cell divisions in a strictly anticlinal fashion, the so-called planar growth. The molecular mechanisms underlying this process are poorly understood. Arabidopsis ovule integuments are excellent model tissues to study planar growth. In our *ucn-1* mutant, localized aberrant cell divisions take place in both the integuments, eventually resulting in multicellular tumor-like protrusions. The *UNICORN (UCN)* gene encodes an active AGCVIII kinase which suppresses ectopic growth in integuments by directly repressing the KANADI transcription factor ABERRANT TESTA SHAPE (ATS) [1, 2]. Furthermore, we could reveal direct interaction between PDK1 (3-PHOSPHOINOSITIDE DEPENDENT KINASE1) and UCN in yeast, *in vitro* and in BiFC assays in protoplasts. The *pdk1 ucn-1* double mutants show rescue of *ucn-1* floral and integument phenotypes. Thus, we conclude UCN-mediated PDK1 repression. In kinase assays, we could show that UCN phosphorylates the PDK1s, leading to the hypothesis that UCN represses PDK1 in a similar but more global fashion compared to ATS.

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[P8] Functional analysis of pollen-specific RALFs during reproduction in maize

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Small secreted peptides can be classified into two major groups, CRPs (cysteine-rich peptides) and non-CRPs. Previous studies showed that multiple CRPs are involved in different steps of the double fertilization process of flowering plants (1). To investigate the roles of CRPs during reproduction in maize, we performed RNA-seq analysis to identify CRPs with specific expression pattern during pollen tube growth and fertilization. We identified three genes encoding rapid alkalinization factors (RALFs, a CRP subgroup), which are highly and exclusively expressed in germinated pollen tubes. Functional studies of RALFs in *Arabidopsis thaliana* revealed that peptides of this gene family were involved in multiple aspects of plant growth and development (2). For example, it has been shown that RALFs interact with FERONIA in Arabidopsis root development and immune signaling (3). Based on sequence alignment and expression pattern comparisons, several putative FERONIA homologs were found to be expressed in maize silks in a pollination-specific expression manner. To understand the function of the pollen-specific RALFs during reproduction in maize, *RALF*-RNAi lines were generated. During *in vitro* germination tests, pollen tubes from down-regulated lines appeared less stable and burst much faster compared with wild type pollen tubes. Transmission efficiency of RALF-RNAi mutants via the male gametophyte was also significantly affected. The effect of pollen cell wall instability and its consequence as well as the specificity of RALF interaction with maize FERONIA homologs is now investigated in more detail both *in vivo* and *in vitro*.

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[P9] Functional analysis of CRPs during reproduction and defense responses in maize

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Double fertilization is a unique sexual reproduction process restricted to angiosperms (flowering plants). Two sperm cells have to be transported to the female gametophyte via a pollen tube through female tissues and fuse with egg cell and central cell to generate embryo and endosperm, respectively. To accomplish double fertilization, extensive male-female communication and accurate guidance is needed at many steps along the pollen tube journey. Previous studies in different plant species revealed that various highly specific receptor-ligand binding activities are critical to accomplish cell-cell communication between the pollen tube and various tissues of the female flower organ. Especially small secreted peptides of various CRP classes appear to play key roles as signaling ligands during pollen tube growth and fertilization (1).

We are using maize as a model to study germline development, pollen tube growth and guidance as well as fertilization mechanism in cereals (2). RNA-seq data of various tissues and cell types indicates that more than 400 CRPs show specific expression pattern during fertilization and pollen/fungal invasion. To further elucidate the biological functions of candidate CRPs during reproduction and defense responses, a number of RNAi transgenic plants were generated with Agrobacterium-mediated stable transformation. For example, a CRP subgroup restricted to C4 grasses appears as pollen tube interactor of the egg apparatus-secreted guidance peptide EA1. We will report and discuss these and other functional studies of CRPs to provide a deeper insight into the understanding of the different roles of CRPs during fertilization and stress response, their evolution and their role in speciation (reproductive isolation) in maize and plants in general.

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[P10] Temperature stress-induced male sterility during pollen development in maize

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Increased temperatures events caused by climate change are becoming more frequent lately. Shift in the duration and intensity of the temperate season is expected to have a detrimental effect on plant development and more drastically in the reproductive development. Stress-induced losses in yield is commonly associated with different physiological, morphological and phenological alterations including changes in flowering initiation, reduced pollen viability and germination, altered embryo development and reduced pistil acceptance. In plant reproduction, male gametogenesis is generally considered the main focus of stress susceptibility. To elucidate the mechanistic basis of heat sensitivity and reduced male gametophyte viability when developing pollen is submitted to heat stress, we imposed a moderate (35°C) heat stress treatment on developing pollen at various stages including the tetrad stage (1). Heat stress resulted in less pollen grains adhered to anthers, reduced pollen viability and germination capability, and pollen grains contained less starch. Our results indicated that alterations in pollen starch composition and the associated reduction in pollen germination strongly correlate with reduced transcriptional activity of starch and soluble sugars degradation enzymes that were strongly downregulated in heat stressed pollen. Thus, although also other stages in pollen development frequently show a negative response on abiotic stress, the tetrad stage as a key developmental process delimitating mitosis and meiosis appears to be one of the most critical developmental stage underlying heat stress sensitivity.

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[P11] Similarities between reproductive and immune processes in the pistil of *Arabidopsis* species

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Extracellular small cysteine-rich peptides, receptor-like kinases and other genes thought to be involved in plant-pathogen interaction and defense also play crucial roles during plant reproduction. Some genes even appear to be involved in both processes. Our study systematically identifies such candidate genes by characterizing the shared and unique differentially expressed genes of the pistil transcriptome during fungal infection and pollination[1].

The comparative analysis of pistils from several *Arabidopsis* species interacting either with own or interspecific pollen or infected by *Fusarium* yielded results and datasets interesting for a diverse group of plant scientists investigating signaling during fertilization, the molecular basis of speciation or *Fusarium* Head Blight (FHB), one of the most devastating plant diseases worldwide.

Our study found pistil transcriptomes responding to fungal infection and growing pollen tubes share a large and informative number of overrepresented genes. Particularly interesting is that *Fusarium* proliferation and pollen tube growth downregulate a large number of genes associated to pathogen killing, most of them encoding defensin-like peptides[2]. The novelty of this observation and its evolutionary conservation make it significant for those investigating the consequences of fungal infection in pollination and seed set.

The largest number of differentially expressed genes occurred in fungal infection. The conserved downregulation of genes involved in cell division and development pointed at a large number of candidates to investigate growth inhibition triggered by immunity and seed set reduction after FHB infection.

Differential gene expression exclusive to pollination showed that in *Arabidopsis thaliana* pistils a large group of thionins and defensins, generally associated to antimicrobial responses, were upregulated after exposure to foreign pollen. This exciting result is relevant for investigating the mechanisms that mediate interspecific pollen rejection and lead to prezygotic reproductive isolation, a topic central to understand speciation.

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[P12] A membrane proteomics approach to identify gamete-expressed cell surface proteins involved in double fertilization

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The fusion of two functional sperm cells with two female reproductive cells (egg cell and central cell) is a characteristic feature of flowering plant sexual reproduction (termed double fertilization) and it generates the two major parts of a seed, the embryo and the endosperm. Although grain yield is one of the important agronomic traits, the molecular players necessary for successful gamete recognition, adhesion and fusion are largely unknown. So far, only three proteins are reported in *Arabidopsis thaliana* to act on the gamete surfaces to accomplish double fertilization: the two essential sperm cell plasma membrane proteins GEX2 (GAMETE-EXPRESSED 2) and GCS1/HAP2 (GENERATIVE CELL SPECIFIC1/HAPLESS 2) are required for gamete adhesion and fusion during fertilization (1,2), while the family of small cysteine-rich EC1 (EGG CELL 1) proteins is secreted by the egg cell upon sperm cell delivery to rapidly activate the sperm cells, indicated by a shift of GCS1/HAP2 from the sperm endomembrane system to the plasma membrane (3).

We aim to learn more about the membrane protein composition of flowering plant gametes. To assess the complexity of the sperm membrane proteome and to identify cell surface proteins potentially involved in male-female gamete interactions we established a method to isolate large quantities of maize sperm cells and performed membrane protein extractions followed by high-throughput proteomics. We will present results of this approach and of our studies on selected candidates and their putative *Arabidopsis* orthologs which are currently being analyzed for their expression pattern and their function during double fertilization using the CRISPR/Cas9 system.

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[P13] Function of AMSH3 and SH3P2 in intracellular membrane trafficking.

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Intracellular membrane transport, including autophagy and vacuolar trafficking, is an essential process for various aspects of plant physiology. Posttranslational modification by ubiquitin is an important signal for diverse cellular processes. Ubiquitinated cargo proteins are recognized by endosomal sorting complexes required for transport (ESCRT) I, II, and III, and hence their delivery to the vacuole for degradation. Ubiquitination is a reversible process, in which ubiquitin conjugates can be hydrolyzed by deubiquitinating enzymes (DUBs). DUBs contribute thus to the maintenance of free ubiquitin pools and regulate the degradation and stability of a target protein.

We are interested in a class of metalloprotease DUBs, namely the AMSH protein family. To understand the molecular mechanism by which AMSH proteins are regulating these intracellular trafficking events, we aimed to identify AMSH-interacting proteins. We have previously shown that AMSH proteins interact with subunits of ESCRT-III and that they are involved in the endocytic - and autophagic protein degradation pathway.

Through further attempts we identified an Arabidopsis SH3 domain containing protein as an interactor of AMSH3. SH3P2 was reported to be involved in autophagy and it interacts with the protein FYVE1/FREE1 that was shown to be involved in the intracellular membrane trafficking pathway and in vacuolar biogenesis. Our analyses showed that SH3P2 is associated with clathrin coated vesicles, binds to ubiquitin and interacts with ESCRT-I. These qualifies SH3P2, to function as an ubiquitin adaptor protein, which recognizes ubiquitinated cargo proteins in the intracellular membrane trafficking and leads them to the ESCRT machinery. We are currently conducting further analyses with the aim to understand the molecular function of SH3P2 and AMSH proteins in intracellular membrane trafficking.

[P14] Identification of ubiquitinated cargo proteins in the intracellular trafficking pathway

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Plant vacuoles are central organs for the storage of proteins, ions and metabolites and the seclusion of salts and heavy metals from the plant cell. They are also involved in the degradation of proteins. Selective protein degradation is important for many processes such as hormone signaling and protein trafficking that regulate plant growth and development. Most of the proteins targeted for selective protein degradation are posttranslationally modified by ubiquitin, which is regulated by ubiquitinating- and deubiquitinating enzymes. AMSH (Associated molecule with the SH3 domain of STAM) is a deubiquitinating enzyme that is a member of the JAMM (JAB1/MPN/MOV34) protease family. The knockout of AMSH1 and AMSH3 leads to severe growth defects and early senescence (1). Our previous analyses have shown that AMSH proteins are involved in endocytic- and autophagic protein degradation. Mutants of *amsh* and *amsh* interactors are vacuolar fusion defective and accumulate ubiquitinated proteins (2). It is assumed that AMSH is required for the degradation of membrane proteins. With the aim to understand the nature of the proteins regulated by AMSH, we now want to identify the ubiquitinated membrane proteins that are accumulated in *amsh* mutants.

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[P15] Points of crosstalk and signal transduction from a systematic experimental phytohormone network map

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In plants all developmental decisions and stress responses are mediated by plant hormones (phytohormones), which bind to intracellular receptors and thereby initiate complex and interconnected signaling events. While principles of plant signal transduction have been revealed over the past decades, a systems perspective is still missing and many components mediating signal transduction and crosstalk remain to be discovered. Here we present an initial and systematic map of the phytohormone signaling network for the reference plant Arabidopsis thaliana.

1,200 protein targets for protein interaction network mapping were selected based either on direct genetic evidence or on membership in a plant family that contains many phytohormone-signaling proteins. Of these, ORFs for about half were available, whereas the remaining 50% needed to be cloned from plasmid template or cDNA, which was achieved with a 97% success rate. Subsequently, these targets were screened three times against each other using an established Y2H based high-quality protein-protein interaction mapping pipeline, that has previously been used to produce a first experimental map of a plant protein interaction network (1). In addition, all 1,200 signaling proteins were screened once against the 12,000 full length Arabidopsis ORFs. These experiments yielded 475 and 698 interactions, after four-fold verification. These interactome data will be validated using an orthogonal assay, which will be benchmarked using a phytohormone specific positive and random reference sets (PRS_{PHY}/RRS_{PHY}) which was also used to test the assay sensitivity in the Y2H-system.

For several signaling pathways, hormone binding to a receptor results in altered protein interactions. To identify these initial signaling events, interaction screening was performed in the presence of hormone to reveal downstream partners of several receptors. These regulated interactions will be integrated with the reference map to infer novel signaling pathways, identify points of cross-talk, and obtain a systems-level perspective onto phytohormone signal transduction plants.

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[P16] Systematic analysis of an experimental phytohormone network map

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Plants are important for human sustenance as source for food, fuel and fiber. Due to man-made climate change plants are threatened by more extreme abiotic and biotic stress conditions. Phytohormones control plant development and mediate responses to environmental conditions and various stress factors. To understand phytohormone signaling pathways and signaling integration a systematic experimental phytohormone network map using a Y2H mapping pipeline was compiled. Therefore 1,200 hormone related proteins were screened for protein-protein interactions to build a PhyHormInteractome (PHI) (1).

Literature curated interactions (LCI) derived from IntAct (3) implied that proteins involved in the same phytohormone signaling pathway show strong interactions, whereas proteins involved in different hormone signaling pathway have only weak interactions. The systematic PHI on the other hand shows almost the same connectivity within hormone signaling pathways than between different pathways. Additionally, the network structure of the literature interactome does not fit the observation, that biological networks have a scale-free or hierarchical network structure (5). The analysis of the network structure of our phytohormone interactome, reveals a hierarchical structure of the network, as in the Arabidopsis interactome AI-1_{MAIN} (2).

For the PHI and the LCI we used a community detection algorithm (4) to identify functional modules. We tested these modules for enrichment in their hormone annotation. This revealed a functional module for seven hormone signaling pathways and a strong connectivity between the modules compared to the LCI. A network randomization shows that the enriched functional modules can be only found in the real network.

Furthermore, we are analyzing under which conditions and in which organs PHI-interactions take place, and where in the interactome we can observe adaption to the environment.

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[P17] Comparative growth response to drought stress in closely related Brassicaceae.

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Abiotic drought and salinity stress can cause severe crop losses and climate change is projected to increase their prevalence in future decades. Extremophile plants, are able to grow in hard environmental conditions and these are therefore of interest to understand plant stress response mechanisms; especially given the limitations of *Arabidopsis thaliana* (*Ath*), which is generally abiotic stress sensitive (1). The evolutionary proximity of extremophile *Ath* relatives like *Arabidopsis lyrata* and *Eutrema salsugineum* makes them good model systems to study adaptive stress response mechanisms. While their ability to survive extreme soil salinity is well investigated (2, 3), their respective drought stress responses and differences to *Ath* are not well characterized.

We performed a side-by-side comparison of the phenotypic responses of these three species during drought stress. We used an automated phenotyping robot that allows precise irrigation and monitoring of different growth parameters in a non-destructive manner. Our results show that, there is an early and a progressive decrease of total growth in all species. Cellular analysis of detached leaves at the end of the experiment showed that this decrease is due to a reduction in cell area; the stomatal index was not affected. Interestingly, inhibition of the photosynthetic machinery was not perceived at earlier stages of the drought treatment, but only when the plants presented a wilting phenotype. So far we did not detect major phenotypic differences in the aerial part of the plants. Despite rewatering experiments revealed a strikingly enhanced survival rate of both, *Arabidopsis lyrata* and *Eutrema salsugineum* in comparison to *Ath.* To better understand the basis of this differential stress tolerance we are currently studying the transcriptomic responses and protein network connectivity of these three brassicaceae.

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[P18] Characterization of LOS2/AtMBP-1 function and activity in *Arabidopsis thaliana*

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Plants as sessile organism are exposed to various environmental conditions, such as cold and freezing temperatures. In order to deal with these abiotic effects, plants have developed various strategies to cope with low temperatures. The best studied signaling cascade is the ICE/CBF-dependent pathway which eventually coordinates the expression of a subset of cold responsive genes, the CBF-regulon[1]. However, in *Arabidopsis thaliana* CBF-independent signaling components under cold stress were also identified, one of them being the *LOS2* locus[2]. *LOS2* is bifunctional, encoding the enolase ENO2, as well as the transcriptional suppressor AtMBP-1 by alternative translation[3], with the latter being an important factor in early cold signaling and ENO2 feedback repression[4]. We aim to further study the role of AtMBP-1 in Arabidopsis not only in cold stress but also in other developmental processes. However, due to the bifunctionality of the locus it is challenging to dissect the roles of both proteins. Therefore, the initial approach involved generating an AtMBP-1 loss-of-function line by mutant rescue. In a second approach we now intend to specifically alter the alternative start codon using a targeted CRISPR/Cas9 approach. The generated mutants will be used in further studies to phenotypically determine the function of AtMBP-1 in different processes. In addition, we aim to identify targets of AtMBP-1 and study the DNA binding behavior of this transcription factor in detail.

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[P19] Characterization of a CESTA orthologue in *Solanum lycopersicum*

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Brassinosteroids (BR) are steroidal plant hormones which are mainly produced in dividing tissues and contribute significantly to the regulation of development and growth in different plant species. Research in the past years has elucidated different molecular mechanisms with which BR signalling contributes to various processes including biotic and abiotic stress responses and the regulation of developmental switches.

CESTA/HALF FILLED (CES/HAF) is a bHLH transcription factor which is involved in BR signalling in *Arabidopsis thaliana*. It was identified through the characterization of a dominant over-expression mutant. Among other traits, the mutant displays a late-flowering phenotype and misshaped leaves[1]. Triple knock-out mutants of CES and its close homologues, BEE1 and BEE3, show defects in silique filling[2]. CES protein localization is regulated by posttranslational modifications, such as phosphorylation by the kinase BIN2, and SUMOylation[3].

Solanum lycopersicum (tomato) is commonly used as a model organism for fleshy fruit development. Beside its economic relevance and dietary benefits, the release of the full genomic sequence of the cultivar 'Heinz 1706' in 2012 facilitated the application of molecular approaches in this species. This project aims to characterize a CES orthologue (SlCES) of tomato and to reveal its role in developmental and physiological processes. The work includes the generation of SlCES gain- and loss-of-function mutants, by combining classic tissue culture techniques with the novel CRISPR/Cas method, and their phenotypic characterization.

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[P20] Parental DNA methylation states are associated with heterosis in Arabidopsis epigenetic hybrids

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Despite the importance and wide exploitation of heterosis in commercial crop breeding, the molecular mechanisms behind this phenomenon are not completely understood. Recent studies have implicated changes in DNA methylation and small RNAs in hybrid performance, however, it remains unclear whether epigenetic changes are a cause or consequence of heterosis.

Here, we present pilot data of a panel of over 500 A. thaliana epigenetic hybrid plants (epiHybrids), which we derived from near-isogenic but epigenetically divergent parents. This proof-of-principle experimental system allowed us to quantify the contribution of parental methylation differences to heterosis. We measured traits such as leaf area (LA), growth rate (GR), flowering time (FT), main stem branching (MSB), rosette branching (RB) and final plant height (HT) and observed several strong positive and negative heterotic phenotypes among the epiHybrids. Using an epigenetic quantitative trait locus mapping approach, we were able to identify specific differentially methylated regions (DMRs) in the parental genomes that are associated with hybrid performance. Sequencing of methylomes, transcriptomes and genomes of selected parent-epiHybrid combinations further showed that these parental DMRs most likely mediate remodeling of methylation and transcriptional states at specific loci in the hybrids. Taken together, our data suggest that locus-specific epigenetic divergence between the parental lines can directly or indirectly trigger heterosis in Arabidopsis hybrids independent of genetic changes.

We are currently scaling up this experimental approach by generating hundreds of epiHybrids families, derived from varying epigenomic backgrounds. High-throughput phenotyping along with population-level methylome sequencing will provide unprecedented insights into role of DNA methylation in hybrid performance in A. thaliana.

[P21] Specificity of combinatorial interactions between abscisic acid receptor components

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The phytohormone abscisic acid (ABA) is induced in response to abiotic stress to mediate plant acclimation to environmental challenge. Key players of the ABA signaling pathway are the ABA-binding receptors (RCAR/PYR1/PYL) which, together with a plant-specific subclade of protein phosphatases 2C (PP2C), form functional holo-receptors. The Arabidopsis genome encodes nine PP2C co-receptors and fourteen different RCARs, which can be divided into three subfamilies. The presence of these gene families in higher plants points to the existence of an intriguing regulatory network and poses questions as to the functional compatibility and specificity of receptor-co-receptor interactions.

We analyzed all RCAR/PP2C combinations for their capacity to regulate ABA signaling by expression in Arabidopsis cells. 113 of 126 possible RCAR-PP2C pairings were found to be functional. The three subfamilies within the RCAR family showed different sensitivities to regulate the ABA response at basal ABA levels when efficiently expressed. At exogenous high ABA levels, the RCARs regulated most PP2Cs and activated the ABA response to a similar extent. The PP2C AHG1 was only regulated by RCAR1/PYL9 to RCAR3/PYL8, which are characterized by a unique tyrosine residue. Site-directed mutagenesis of RCAR1 showed that its tyrosine residue is critical for AHG1 interaction and regulation. Furthermore, the PP2Cs HAI1 to HAI3 were regulated by all RCARs. The findings unravel the interaction network of possible RCAR-PP2C pairings and their different potentials to serve a rheostat function for integrating fluctuating hormone levels into the ABA response pathway.

[P22] Leveraging abscisic acid receptors for water productivity in Arabidopsis

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Plant growth requires the influx of atmospheric CO_2 through stomatal pores, and this carbon uptake for photosynthesis is inherently associated with a large efflux of water vapour. Under water deficit plants reduce transpiration and are able to improve carbon for water exchange leading to higher water use efficiency (WUE). Whether increased WUE can be achieved without trade-offs in plant growth is debated. The signals mediating the WUE response under water deficit are not fully elucidated but involve the phytohormone abscisic acid (ABA). ABA is perceived by a family of related receptors mediating acclimation responses and reduced transpiration. We now show that enhanced stimulation of ABA signalling via distinct ABA receptors can result in plants constitutively growing at high WUE in the model species Arabidopsis. Furthermore, plants expressing the ABA receptors RCAR6/PYL12 combined up to 40% increased WUE with high growth rates, i.e. are water-productive. Water productivity was associated with maintenance of net carbon assimilation by compensatory increases of leaf CO_2 gradients thereby sustaining biomass acquisition. Leaf surface temperatures and growth potentials of plants growing under well-watered conditions were found to be reliable indicators for water productivity. The study shows that ABA receptors can be explored to generate more plant biomass per water transpired, which is a prime goal for a more sustainable water usage in agriculture.

[P23] Functional characterization of a genomic region associated with carbon isotope discrimination and WUE in maize

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During photosynthesis plants discriminate against the stable isotope ^{13}C. In plant breeding, this discrimination ($\Delta^{13}C$) is used as a desirable physiological trait associated with water use efficiency (WUE) and drought tolerance. In C_3 plants, $\Delta^{13}C$ is positively correlated with stomatal conductance, a trait which has a major impact on transpiration rate, and has been shown as an accurate predictor for yield under drought. In C4 plants, due to the different nature of carbon fixation, more factors contribute to $\Delta^{13}C$ and the knowledge about its link to WUE and drought tolerance is more limited.

Using a maize introgression library (IL), we identified a genomic region on chromosome 7, which influences $\Delta^{13}C$. Here, we phenotype a near isogenic line (NIL), carrying the genomic region of interest as a single chromosome fragment of the donor parent (DP) in the genetic background of the recurrent parent (RP). The NIL showed significantly decreased $\Delta^{13}C$ compared to the RP, which correlates with an increase in stomatal conductance due to both, altered stomatal aperture and development, decreased abscisic acid content, increased leaf transpiration rate and decreased WUE. We identified several promising candidate genes situated in the chromosome fragment and we are currently functionally characterizing them through physiological and molecular approaches.

[P24] Is auxin signaling part of the RAM1-regulated arbuscocyte developmental program?

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The exchange of nutrients in arbuscular mycorrhiza symbiosis between plants and glomeromycotan fungi is performed by fungal broccoli-shaped structures, called arbuscules (1,2). *REDUCED ARBUSCULAR MYCORRHIZA1* (*RAM1*), encoding a GRAS transcription factor, was identified to be a core regulator of arbuscule development (3,4,5). The *ram1* mutant displays a stunted arbuscule phenotype (3,4,5). Arbuscule branching also requires auxin signaling (6). We wondered how auxin signaling is placed relative to RAM1 in a signaling network regulating arbuscule development. We found in *L. japonicus* that the auxin reporter *DR5*:*GUS* was active in arbuscocytes (arbuscule containing cells) in the wild type but not in *ram1* indicating that *RAM1* is required for *DR5*:*GUS* induction in these cells. Furthermore, ectopic expression of *RAM1* induced *DR5*:*GUS* in a patchy pattern in absence of the fungus. In addition, some AM-induced auxin response genes were not induced in the *ram1* mutant. Taken together, this indicates that activation of auxin signaling or biosynthesis in arbuscocytes may involve RAM1.

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[P25] The development of hyphal symbionts and pathogens relies on fatty acid biosynthesis by the plant host

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Many biotrophic filamentous microbes develop host membrane-surrounded hyphal structures, such as haustoria or arbuscules inside plant cells. The development of these putative feeding structures depends on a genetic program of the host plant that allows compatibility and mediates cellular rearrangement for intracellular accommodation of the hyphal structure. In a forward genetics screen designed to find *Lotus japonicus* mutants that are perturbed in arbuscular mycorrhiza development we identified the mutant *disorganized arbuscules* (*dis*) that is impaired in arbuscule branching. We identified the causative mutation in the *DIS* gene, which encodes an enzyme involved in fatty acid elongation, of which three paralogs are present in the *Lotus japonicus* genome. The *DIS* promoter is active in arbuscule containing cells. A DIS-YFP-fusion was targeted to plastids, consistent with the subcellular localization of fatty acid biosynthesis. The simultaneous presence of KASI gene related to housekeeping function and a symbiosis-related paralog (DIS) is conserved in AM-forming dicotyledons. In contrast, the genomes of eight plant species, which are unable to form AM, contain only a single KASI gene. A *kasI* mutant of *Arabidopsis thaliana* impairs development and reproductive success of the biotrophic pathogens *Hyaloperonospora arabidopsidis* (oomycete) and *Erysiphe cruciferarum* (ascomycete). Taken together these data indicate that host fatty acid biosynthesis might be a critical compatibility factor for mutualistic and parasitic interactions of plants with biotrophic filamentous microbes.

[P26] Identifying the targets of RAM1, a putative transcriptional regulator of arbuscular mycorrhiza symbiosis

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Arbuscules are the most important interface for mutualistic nutrient exchange in the arbuscular mycorrhiza (AM) symbiosis, formed between most land plants and the fungi of the phylum of Glomeromycota (1). These are microscopic, tree-shaped fungal structures, formed in cortical cells of colonized roots. Arbuscule formation is under the control of the host plant and the *Lotus japonicus* mutant *ram1* exhibits formation of stunted arbuscules, a lowered colonization level and a reduced expression of marker genes for arbuscule development (2). The *RAM1* gene encodes a GRAS protein and is upregulated exclusively during AM symbiosis (3). When overexpressed in the absence of fungal colonization it is capable of inducing AM marker genes associated with arbuscule formation (2). Therefore, it is very likely that RAM1 plays a crucial role as transcriptional regulator in the AM symbiosis, in particular during arbuscule development. However, the complete set of targets is currently unknown although it would provide important knowledge about the complex molecular processes involved in arbuscule formation. Therefore, we chose a trancriptomics approach to determine the target genes of RAM1. Our strategy involves ectopic, controlled induction in the absence of the symbiotic partner, followed by an RNA-sequencing analysis to determine differential expressed genes upon *RAM1* induction. To this end, we are currently testing different genetic systems allowing inducible gene expression in plants.

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[P27] A CCaMK-CYCLOPS-DELLA complex activates transcription of *RAM1* to regulate arbuscule branching

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Arbuscular mycorrhiza (AM) symbiosis is the most widespread strategy of plants to acquire mineral nutrients from soil. Root colonization by AM fungi culminates in the formation of highly branched structures called arbuscules that releases the mineral nutrients to the plant. The regulation of number and branching status of arbuscules is poorly understood, which might influence symbiotic nutrient-transfer efficiency. To identify molecular players in arbuscule development a forward genetic screen was performed and identified the *Lotus japonicus* mutant *reduced and degenerate arbuscules (red)*, which is impaired in arbuscule branching. Using mapping and next generation sequencing (NGS) approach, we identified the causative mutations in *red* perturbed a gene encoding the GRAS protein REDUCED ARBUSCULAR MYCORRHIZA 1 (RAM1). Here we conduct an epistasis analysis to place *RAM1* relative to the symbiosis (CCAMK-CYCLOPS) and GA signaling (DELLA). A nuclear calcium spiking is triggered upon perception of mycorrhiza factor, which is then decoded by a nuclear localized calcium and calmodulin dependent kinase (CCaMK). CCaMK then interacts and phosphorylates the transcription factor CYCLOPS that activates downstream AM signaling. Ectopic expression of *RAM1* not only complements *cyclops* mutants but also could support arbuscule development in presence of suppressive gibberellin. These results places *RAM1* downstream of *CCaMK*, *CYCLOPS* and *DELLA*. We further show that CCaMK-CYCLOPS-DELLA forms a complex that activates *RAM1* expression via binding of CYCLOPS to a *cis*-element in the *RAM1* promoter. Thus, the *RAM1* promoter is an integral node for symbiosis (CCaMK-CYCLOPS) and GA signaling (DELLA).

[P28] Karrikin signaling in *Lotus japonicus*

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About 80% of land plant species form arbuscular mycorrhizal (AM) symbiosis with fungi of the phylum *Glomeromycota* (1). While AM fungi are obligate biotrophs depending on this ancient symbiosis to survive (2), the plants benefit from an increased nutrient availability and resistance to biotic and abiotic stress (3; 4; 5). Before physical contact strigolactones, recently discovered plant hormones, and fungal signaling molecules called Myc factors initiate signaling between the partners. Then the fungus forms attachment structures called hyphopodia on the root surface, penetrates the root tissue and finally develops branched arbuscules inside roots cortex cells (6).

Recently D14L a homolog of the *Arabidopsis thaliana* karrikin receptor (KAI2), was shown to be involved in AM colonization in rice (7). Karrikin, a signaling molecule derived from burned plant material (8), is structurally related to the strigolactones and the perception of both molecules share similar mechanisms. The strigolactone receptor D14 and the karrikin receptor D14Like are members of the α/β hydrolase family and they are supposed to interact with the F-box protein MAX2, which is part of a Skp1–Cullin–F-box (SCF) ubiquitin E3 ligase complex. After signal perception the SCF-complex targets repressors of either strigolactone or karrikin signaling responses to degradation by the proteasome via ubiquitiniation. In rice D53 was identified as a repressor of strigolactone signaling, while a homologues protein in *Arabidopsis thaliana* SMAX1 turned out to be a repressor of karrikin signaling (9; 10; 11). We are interested in the molecular mechanisms of strigolactone and karrikin signaling in *Lotus japonicus* during AM symbiosis. Therefore, we aim at identifying receptor complex dynamics and additional complex components by expressing suitable tagged versions of the α/β hydrolases D14, D14La and D14Lb, and the F-box protein MAX2 to perform expression analyses and co-immunoprecipitations followed by Mass Spectrometry under various conditions.

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[P29] Role of KAI2 in root growth direction

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The *Arabidopsis thaliana KARRIKIN INSENSITIVE 2* (*KAI2*) is the putative receptor of karrikin, a butenolide compound found in smoke that can promote seed germination in fire-following plants (1). KAI2 mediated signaling has been recognized as regulator of plant development (2, 3). The *kai2* mutant exhibits delayed germination, *kai2* seedlings have an elongated hypocotyl and epinastic cotyledons. During vegetative development, *kai2* leaves are elongated with curled margins (4). However, the role of *KAI2* in root development is still unknown. Analysis of root behavior is traditionally carried out after germinating seedlings on a hard agar surface in a petri dish. In these conditions the root cannot penetrate the agar, causing morphological changes such as root skewing (5). Skewing was initially described in *Arabidopsis thaliana* wild type roots of the ecotype *Landsberg erecta* (*Ler*) as the tendency of the root to deviate their growth progressively away from the vertical, always as right-slanted when viewed from the bottom of the plate (6). We observed that the Arabidopsis *kai2* mutant displays exaggerated skewing and we are currently characterizing the molecular basis of this response.

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[P30] The membrane nanodomain-associated receptor-like kinase RIR1 contributes to resistance in *Arabidopsis thaliana*

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Pathogen-Associated Molecular Patterns (PAMPs) are recognized by Pattern Recognition Receptors (PRRs), which are central components of multiprotein complexes at the plasma membrane (PM). To trigger an immune response, the compartmentalization of the membrane is crucial for cell signalling. REMORIN (REMs) proteins are molecular scaffolds in response to abiotic and biotic stress, and are the best-characterized PM-domain markers in plants. However, the functionality and molecular mechanisms of REM PM-nanodomain in living cells needs to be further clarified. Here, we identified the *Arabidopsis thaliana* REMORIN-INTERACTING RECEPTOR 1 (RIR1), a malectin-domain containing leucine-rich repeat receptor-like kinase (RLK) to interact with the molecular scaffold protein remorin REM1.2. A combination of live cell imaging approaches enabled us to show that RIR1 localizes to and preferentially interacts with remorins in distinct membrane nanodomains. Functionally, REM1.2 restricts overall lateral mobility of the receptor at the plasma membrane. *rir1* mutants are more susceptible to different biotrophic pathogens and show significantly decreased levels of callose depositions in leaves. This callose phenotype coincides with a temporally controlled REM1.2–independent recruitment of the RLK to the haustorial encasement membrane where it co-localizes with remorins and callose in a temporally controlled manner. RIR1 represents a novel receptor-like kinase that contributes to resistance and callose deposition in *A. thaliana*.

[P31] The role of group 1 remorins in response to abiotic stress

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Plant growth and development are highly affected by abiotic stress including temperature changes, drought and salinity of the soil. Increasing evidence suggests that cellular integration of the corresponding signals occurs within pre-formed hubs at the plasma membrane called ‘nanodomains’. These membrane sub-compartments are most likely organized by multivalent molecular scaffold proteins, such as flotillins and remorins. Latter form a plant-specific multigene family with six subgroups. They associate with the inner leaflet of the plasma membrane and contain an intrinsically disordered segment in the N-terminal region, which provides the remorin proteins with the ability to interact with multiple partner proteins. Our data indicate that at least three group 1 remorins form a heteromeric complex in the plasma membrane, which may facilitate the molecular assembly of functionally specified signaling hubs that function in biotic and abiotic stress responses. Our data indicates direct interaction between an undescribed receptor kinase and AtREM1.2 but not AtREM1.3, suggesting different interactors for the remorins within the complex.

Furthermore, systematic phenotypical analyses of single, double and triple mutants revealed an increased sensitivity towards drought and an altered cell wall composition. A large scale interactome approach should now help to identify new interaction partners and molecular pathways that modulate plant abiotic stress responses.

[P32] Transgenic expression of the maize benzoxazinoid biosynthesis pathway in *Arabidopsis thaliana*

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Secondary metabolites constitute the chemical defence arsenal of plants. Specific defence compounds out of a reservoir of hundreds of thousands are character-istically found in specific plant families. Benzoxazinoids are plant defence compounds mainly produced by grasses. The main benzoxazinoids (BXs) DIBOA and DIMBOA, are synthetized in the seedling and confer resistance against pathogens and herbivores. BX biosynthesis is connected to the tryptophan pathway, all required genes (*Bx1* to *Bx9*) are known from maize and hence theoretically BX biosynthesis can be expressed transgenically in every plant. To confirm the mode of action of BX and assess the effects of the compounds on plant physiology and development, and on protection against herbivores and microbial pathogens we are working on a transgenic expression of the whole biosynthesis pathway in *Arabidopsis thaliana*, a dicot with no endogenic BX production.

All maize enzymes of DIBOA biosynthesis, the indole synthase BX1, four cytochromes P450s (BX2 to BX5) and a cytosolic UGT (BX8), could be functionally expressed in *S. cerevisiae* and *A. thaliana*. The expression of the whole pathway chain however so far could not produce the final biosynthesis product DIBOA. In HPLC and LC-MS analyses intermediates of the BX pathway have shown to be readily modified, e.g. by glycosylation in *A. thaliana* and *N. benthamiana*. To avoid such modifications of xenobiotics *in planta,* intermediates have to be captured. This might take place in a protein complex called metabolon. Such a metabolon hypothe-tically exists in maize. The P450-oxidoreductase is a known nucleation site and the soluble UGT *Bx8* could also contribute to the complex formation. The impact of these proteins for BX biosynthesis is currently investigated in *A. thaliana* and *S. cerevisiae*. For the evolution of secondary metabolic pathways (ER-)membrane domains that recruit enzyme complexes might be essential: the diffusion of toxic intermediates will be reduced and at the same time the efficiency of the catalysis will be increased.

[P33] Convergent evolution of secondary metabolite pathways in the dicots *Lamium galeobdolon* and *Consolida orientalis*

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Benzoxazinoids are secondary metabolites widespread in the grasses. They also occur in some dicotyledonous species such as *Lamium galeobdolon* of the Lamiaceae and *Consolida orientalis* of the Ranunculaceae. Their biosynthetic pathway is well established in maize, within the dicots it is best elucidated for *C. orientalis*. There the branchpoint enzyme *Co*BX1, the UDP-glucosyltransferase (UGT) *Co*BX8 and the β-glucosidase (β-GLU) *Co*BGLU have been characterised. Phylogenetic comparisons indicate convergent evolution of these enzymes in monocots and dicots. Moreover, comparison of *Co*BX1 and putative branchpoint enzymes in *L. galeobdolon* show polyphyletic origins in dicots as well. In maize hydroxylation of indole by four cytochrome P450 dependent monooxygenases (P450s) results in the first active compound. We aim to identify benzoxazinoid specific β-GLU and UGT in *L. galeobdolon* and P450s in *C. orientalis* to get further insight into phylogenetic origin of the complete pathway. At present, verification of candidate enzymes is being carried out after heterologous expression in different systems. Preliminary results indicate that the evolution of the benzoxazinoid specific β-GLU is convergent within the dicots. The analysis will shed light on the evolutionary flexibility in recruitment of different ancestors in secondary metabolite pathways.

[P34] Phenotyping plant-microbe-interactions

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Pathogens cause substantial crop losses, challenging modern plant breeding to select crop cultivars that balance yield and (elevated) resistance to disease. Here, we present medium- and high-throughput phenotyping platforms to assess plant growth and disease resistance traits in plants under biotic stress. Root and shoot growth parameters of medium-sized plants can be screened during different stages of plant-pathogen interactions. In parallel, disease resistance traits can be monitored from the molecular to the whole plant level. A newly developed multi-cuvette system allows us to analyze a wide spectrum of volatile organic compounds that are emitted by plants and are considered markers for plant stress and stress resistance phenotypes. Furthermore, pathogen propagation is monitored on a confocal imaging platform that supports the analysis of live seedlings cultivated in multi-well plates (non-invasive) and of leaf discs of mature plants (invasive). Together, this phenotyping pipeline for plant-pathogen interactions integrates plant growth characteristics with robust biotic stress tolerance data sets supporting breeding efforts for high yield, high resistance crop cultivars.

[P35] How WRKY and ETHYLENE RESPONSE FACTORs prime (systemic) immunity in barley

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Systemic acquired resistance (SAR) is a plant defence response that is triggered by a foliar infection and enhances resistance in systemic, uninfected parts of the plant in case of a subsequent pathogen challenge. Many signals and genes involved in SAR have been discovered in *Arabidopsis thaliana*, wherein SAR is associated with salicylic acid (SA)-dependent signalling. In monocotyledonous plants, less is known about SAR and the involved signalling mechanisms. Recently, we showed that in barley (*Hordeum vulgare*) bacteria-triggered systemic resistance to a hemibiotrophic bacterium can be induced (1). Unlike SAR in *Arabidopsis*, this was neither associated with SA nor with NPR1. Interestingly, the same pre-treatment also induced systemic susceptibility to a necrotrophic fungus. Illumina-based RNA sequencing revealed a link between SAR-like immunity in barley and ethylene response factors (ERFs) as well as WRKY transcription factors (TFs) (1).

CRISPR/Cas9-generated knock-out plants will be used to further study the role of these TFs in barley SAR. To this end, mutagenesis efficiencies of CRISPR/Cas9 constructs with different gRNAs targeting one or two *ERF* genes (*HvERF-like* or/and *HvERF4*) were evaluated using a fluorescent reporter system in barley protoplasts. The mutagenesis rates of the different gRNAs showed marked differences, varying between 1 and 35%. In parallel, stable transgenic barley lines expressing CRISPR/Cas9 constructs were generated. One T_0 line with a heterozygous mutation in one of the target sequences was detected, showing that the CRISPR/Cas9 vectors are active in barley plants. More lines with mutations are expected in T_1 and T_2 generations. These mutants will be examined for alterations in induced defence responses.

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[P36] ROP-INTERACTIVE PARTNER b links barley ROP signaling to microtubules and acts downstream of the powdery mildew susceptibility factor RACB

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Blumeria graminis f.sp. *hordei* (*Bgh*) is a pathogenic fungus causing the powdery mildew disease on barley (*Hordeum vulgare*). In the beginning of its biotrophic lifecycle the fungus has to penetrate the cell wall and establish a specialized feeding structure, the haustorium. During this process, the host cell stays alive and a polar delivery of membrane material can be observed, resulting in the establishment of the extrahaustorial membrane.

Barley RACB is a small monomeric G-Protein of the Rho-of-plants (ROP) class and a susceptibility factor in the interaction of barley with *Bgh* [1]. Transient overexpression of constitutively activated RACB increases susceptibility of barley, whereas silencing of RACB decreases susceptibility to *Bgh*. ROP's function as molecular switches with an active GTP-bound and an inactive GDP-bound stage and are involved in a plethora of signaling processes as well as many polarization processes in plants, for example in root-hair formation and pollen tube elongation [2, 3]. RACB knock-down lines are impaired in root-hair elongation and stomata development, two processes where cell polarization is important [4].

In order to decipher the RACB signaling pathway that *Bgh* exploits it is necessary to find downstream interactors of RACB. We identified the scaffold protein ROP-INTERACTIVE PARTNER b (RIPb) as a potential downstream effector of RACB signaling. RIPb shows interaction with RACB in yeast-two-hybrid and BiFC assays. Localization studies indicate that RIPb might link RACB to the microtubule cytoskeleton and overexpression of RIPb increases susceptibility of barley to *Bgh*.

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[P37] Regulation and stability of the barley susceptibility factor HvRACB

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In barley (*Hordeum vulgare*), the function of the small GTPase HvRACB has been extensively studied in the interaction with the fungal pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*). It has been shown that HvRACB acts as a susceptibility factor, which might be targeted by the fungus to facilitate infection [1]. Recently, we established and verified stably transgenic barley lines expressing constitutively active (CA)-HvRACB containing various tags. With these plants, we purified large amounts of CA-HvRACB protein by immunoprecipitation (IP) in order to analyse potential interaction partners via an unbiased mass spectrometry (MS)-screening. Promising candidates included proteins involved in vesicular trafficking, such as a RAB GTPase and a coatomer subunit. Additionally, we intend to determine the *in vivo* phosphorylation status and site(s) of CA-HvRACB, since we identified the HvRACB-interacting ROP-BINDING PROTEIN KINASE 1 (HvRBK1) in previous experiments [2]. HvRBK1 *in vitro* phosphorylates HvRACB and itself in a CAHvRACB-dependent manner and binds to a type II S-PHASE KINASE 1-ASSOCIATED (SKP1)-LIKE PROTEIN (HvSKP1-like) in yeast and in planta [3]. SKP1 proteins are subunits of the SKP1-CULLIN 1-FBOX (SCF)–E3 ubiquitin ligase complex, which specifically targets proteins for proteasomal degradation via the ubiquitination pathway [4]. RNAi-mediated knockdown of HvRBK1 and HvSKP1-like increased protein abundance of HvRACB, while simultaneously making barley more susceptible to *Bgh* [3]. Hence, we suggest that the susceptibility factor HvRACB, once activated, becomes phosphorylated and a target for ubiquitin-dependent degradation.

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[P38] Various RIC proteins interact with barley susceptibility factor RACB suggesting a role during pathogenesis

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Biotrophic pathogens like the barley powdery mildew fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) require living host tissue during the first stage of infection. The barley ROP (RHO of plants) RACB is a small monomeric G-protein and required for full susceptibility to penetration by *Bgh*, potentially due to its role in cell polarization [1][2]. In eukaryotes in general, RHO (rat sarcoma homologue) GTPases are molecular switches that translate intracellular and extracellular stimuli into downstream responses. Consequentially, active RACB triggers certain signaling pathways by interacting with different proteins. We have shown previously that RACB interacts *in planta* with RIC171 (ROP-interactive CRIB-motif containing protein) and overexpression of RIC171 caused a higher susceptibility of barley epidermal cells to *Bgh* [3]. Interacting with ROP GTPases via its conserved CRIB domain, RIC proteins function as adapter molecules linking ROPs to diverse downstream targets [4]. Here we show, that barley RACB also interacts with RIC proteins 157 and 163 in yeast. Transient overexpression of RIC157, similar to RIC171, rendered barley epidermal cells more susceptible to penetration by *Bgh*. Interestingly, this elevated susceptibility is abolished by simultaneous transient induced gene silencing of RACB. Since RACB seems to be a key player in cytoskeleton organization, we suggest that RIC proteins are involved in pathogenesis by supporting entry of *Bgh* into barley cells, probably also due to their RACB-dependent recruitment to the cell periphery.

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[P39] Generation of lipopolysaccharide biosynthesis mutants of plant-associated Pseudomonas strains

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In order to establish an infection, the plant-associated bacteria have to successfully colonize and invade the plant. While they have to overcome various chemical and physiological barriers as well as defense responses, bacteria rely on various defense features themselves. Lipopolysaccharide (LPS), the major component of gram-negative bacteria outer membrane (OM) directly contributes to permeability and integrity stabilizing properties of the OM. Furthermore, the LPS O-polysaccharide (OPS) is mainly responsible to fend off cationic antimicrobial peptides and other defense agents targeting the bacterial cell envelope. To elucidate the role of OPS in pathogenicity of adapted plant-associated bacteria we seek to generate mutant strains of Pseudomonas syringae pv. tomato (Pst) DC3000 producing truncated LPS molecules. Furthermore, analysis by means of bioinformatic analysis were conducted to elucidate the genetic background facilitating the biosynthesis of LPS structures in phytopathogens. The bioinformatic analysis revealed several conserved homologs of LPS biosynthesis genes, documented in Escherichia coli and Pseudomonas aeruginosa, in the genomes of Pst DC3000 and related plant-associated Pseudomonas strains. By this means we could narrow down conserved as well as missing parts of the synthesis pathway. We further used the informations of the bioinformatic analysis to identify single gene targets to create knockout mutant strains incapable to synthesise OPS. Additionally, generated Pst DC3000 rough LPS strains were then further characterized in swarming motility and pathogenicity assays.

[P40] Analysis of potential downstream signaling components of the receptor-like kinase LORE during lipopolysaccharide perception in *A. thaliana*

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Plants employ a complex innate immune system to detect and combat invading pathogens. Pattern recognition receptors at the plasma membrane perceive conserved structural components of microbes, so-called microbe-associated molecular patterns. This induces pattern-triggered immunity, a broad-spectrum defense response that can ward off most pathogens. The receptor-like kinase (RLK) LORE (lipooligosaccharide-specific reduced elicitation) is a key component for perception of lipopolysaccharides (LPS), an integral part of the cell wall of Gram-negative bacteria (1).

Activation and regulation of RLKs and signal transduction commonly requires the formation of multi-protein complexes which can involve RLKs, receptor-like proteins, and various members of the receptor-like cytoplasmic kinase family (RLCK) or regulatory proteins. The RLCK BIK1 was shown to be a common signaling partner of several classes of RLK, while other RLCKs like PBL1 seem to be associated only with certain classes of RLKs (2). Knock-out of BIK1 greatly reduces LORE-dependent ROS production and Ca^{2+}-ion influx during LPS perception in *A. thaliana*. A loss of PBL1 however slightly reduces LPS-dependent ROS production and has no influence on Ca^{2+}-ion levels. PBL27 was described as a specific signaling component for perception of fungal chitin (2), but a *pbl27* mutant line shows normal LPS-dependent immune responses. Taken together, this highlights the role of BIK1 as common signaling hub, and shows that different RLCKs play differential roles during signaling events of RLKs from different classes.

Signal attenuation and regulation of RLKs can be achieved via calmodulins (CaMs), a class of Ca^{2+}-binding regulators of RLKs as described for the phytosulfokine receptor PSKR1 (3). We could show that LORE contains a CaM-binding motif in the kinase domain and seems to be able to interact with canonical CaMs, suggesting that LORE activity might be regulated via CaMs in a Ca^{2+}-dependent manner.

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[P41] Receptor complex formation and effects of over expression of the receptor-like kinase LORE on innate immunity of *Arabidopsis thaliana*

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Plants evolved molecular strategies to ward off pathogens. Perception of conserved microbe-associated molecular patterns (MAMPs) like lipopolysaccharide (LPS) leads to activation of defence responses in plants. This so-called pattern-triggered immunity (PTI) is mediated by specific pattern-recognition receptors (PRRs) at the cell surface. Binding of MAMPs to the respective PRR triggers several downstream responses, for instance calcium signalling and reactive oxygen species production. PRR signal transduction typically involves the formation and activation of receptor complexes.

Recently, we identified the receptor-like kinase LORE (LipoOligosaccharide-specific Reduced Elicitation) as a key component of LPS signalling in *Arabidopsis thaliana* [1]. LORE is part of the plant-specific class of bulb-type lectin S-domain-1 kinases (SD-RLKs) with 32 members in Arabidopsis and is related to the self-incompatibility conferring S-locus receptor kinase SRK of Brassica. SRK forms homodimers in a ligand-independent manner [2]. Our findings show that LORE is also able to form homodimers in the absence of LPS. Further work aims to elucidate LORE receptor complex formation and to identify extracellular domains essential for receptor dimerization. Additionally, we generated LORE over expression lines and are currently investigating the influence of elevated receptor levels onto typical immune responses and the resistance against *Pseudomonas syringae*.

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[P42] Transcriptional regulation by the CCaMK/CYCLOPS complex is dependent on its phosphorylation status

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Legumes can engage in beneficial endo-symbioses called Root Nodule Symbiosis (with nitrogen fixing bacteria) and Arbuscular Mycorrhiza Symbiosis (with phosphate-acquiring arbuscular mycorrhiza fungi). For the establishment and maintenance of either symbiosis an overlapping set of signaling components (the common symbiosis genes) is required. This signaling cascade includes the DNA-binding transcription factor CYCLOPS that interacts with and is phosphorylated by the calcium and calmodulin dependent kinase CCaMK in the nucleus (1). Upon phosphorylation by CCaMK CYCLOPS activates the transcription of transcription factor genes that are important for Root Nodule Symbiosis, such as NODULE INCEPTION (NIN) (2) and ETHYLENE RESPONSE FACTOR REQUIRED FOR NODULATION1 (ERN1) (3), or for Arbuscular Mycorrhiza Symbiosis, namely REDUCED ARBUSCULAR MYCORRHIZA1 (RAM1) (4). This will ultimately lead to distinct developmental re-programing for the respective symbiosis *in planta*.

Phosphorylation of CYCLOPS at positions S50 and S154 are sufficient for nodule organogenesis and activation of the *NIN* promoter (2). Using quantitative transactivation assays we identified additional phosphorylation sites in CYCLOPS, suggesting that phosphorylation of CYCLOPS plays a regulatory role during the establishment of the Arbuscular Mycorrhiza or the Root Nodule Symbiosis.

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[P43] Lateral root formation is stimulated by dominant-active versions of common symbiosis genes in *Lotus japonicus*

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Plant productivity depends on the adaptation of the root system architecture to uneven nutrient distribution and inhomogeneous soil structure. In addition to abiotic factors, interactions with arbuscular mycorrhiza fungi or lipo-chitooligosaccharide signals from rhizobia or fungi modulate root system architecture by stimulating lateral root formation (1-4). The molecular mechanisms that regulate nodule and lateral root formation have been studied in legumes and in *Arabidopsis thaliana*, respectively, but little is known about the connection between the symbiotic signaling pathway and that of lateral root formation. In order to understand the genetic basis or the molecular connection between the lateral organ developments, we developed a quantitative lateral root induction assay and studied the effect of dominant active version of common symbiosis genes on lateral root organ frequency in *Lotus japonicus*. We observed that spontaneous activation of the nodulation program mediated by dominant active versions of *SYMRK*, *CCaMK* and *CYCLOPS* strongly stimulate lateral roots emergence. Our data support the idea that the development of root nodules evolved by recruiting deviating signaling processes previously established for the regulation of lateral root emergence.

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[P44] The inhibitory role of NIN in nodulation

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During evolution, plants have evolved a variety of mechanisms to overcome deficient nutrient concentrations in the soil. One important strategy is the symbioses with nitrogen-fixing bacteria and arbuscular mycorrhiza fungi, which provides them nitrogen and phosphorus, respectively, in exchange for photosynthetically fixed carbon. The establishment of these symbioses is controlled by a common set of genes and features perinuclear calcium oscillations, conceptually deciphered by CCaMK (1). CCaMK phosphorylates CYCLOPS and forms a protein complex (CCaMK/CYCLOPS) that transcriptionally activates downstream genes required for specific developmental responses in arbuscular mycorrhiza and root nodule symbiosis (1,2). One target of the CCaMK/CYCLOPS complex is *NIN* (nodule inception) (3). NIN is a nodulation specific transcription factor that plays a key role in both infection and nodule organogenesis processes (3). We confirmed that NIN overexpression inhibits nodulation (4); interestingly the inhibition observed was exclusively in transformed roots. An alternative mechanism by which NIN inhibits nodulation is currently under investigation.

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[P45] A small subset of NLR genes drive adaptation in wild tomato

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In plants, defence-associated genes are under constant evolutionary pressure to adapt to the pathogen. Indeed, major defence gene families like the NLR resistance gene family, show stronger positive selection between plant species than others. However, the extend of NLR evolution in shorter time scales remains unclear. We use a three-pronged approach to quantify the adaptation of NLR genes within a single wild tomato species in a demographic context.

We generated a novo genome of *Solanum chilense,* a species endemic to southern Peru and northern Chile with distinct and separated populations. Whole genome resequencing of geographically distant individuals in combination with Monte Carlo simulations established the species demography. By using targeted resequencing we then show that a small subset (7%) of NLR show signs of extreme positive or balancing selection between one or more of 14 populations., generating a mosaic of NLR alleles throughout the species range.

Identification of such diversification events in different habitats allows to place pathogen resistance in an ecological context, linking host, pathogen and climate. Such information is crucial for durable resistance breeding in a world with global climate change.

[P46] Homer´s myth of the Trojan horse meets a small protein in *Zea mays* development

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Plants lack a germ line, thus within the flower adult somatic cells must switch from mitotic proliferation to competence for meiosis. In maize (*Zea mays*) anthers the first step is archesporial (AR) cell specification followed by AR-directed specification of the somatic niche. We have proposed that this process sequentially requires two small proteins, MSCA1 (MALE STERILE CONVERTED ANTHER1) and MAC1 (MULTIPLE ARCHESPORIAL CELLS1). Loss of *Mac1* results in excess archesporial cells, delayed periclinal division of neighboring Layer2-derived cells which normally develop into the somatic niche, and ultimately leads to male sterility. Yet, the precise timing and function of MAC1 remains unclear. So far, thousands more small proteins with unknown roles in maize anther development have been identified by mass spectrometry but not characterized. To analyze these candidates, we were inspired by Homer´s Trojan horse myth and developed a novel system to deliver tagged proteins into maize in a highly localized fashion. A genetically modified version of the biotropic maize pathogen *Ustilago maydis* was used to secret fluorescently tagged MAC1 into the anther apoplast. Using this Trojan horse approach, we could rescue the *mac1*-mutant phenotype, locally where a fungal hypha contacted Layer2-derived cells, indicating that MAC1 serves as a cell-autonomous signal during somatic layer development. This study proves that *U. maydis*, which infects all aerial parts of the maize plant, can be used as a valuable tool to characterize maize proteins *in vivo* without genetical modification of the plant.

[P47] Cross-kingdom RNA interference is widespread in microbial pathogens to suppress plant innate immunity

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Fungal small RNAs (sRNAs) suppress plant immunity by hijacking host RNA interference (RNAi) pathways. The grey mold fungal pathogen *Botrytis cinerea* delivers sRNAs into the plant cells, which hijack the plant Argonaute (AGO)/RNA induced gene silencing complex to suppress host immunity genes (1,2). It is currently not known whether such pathogenic cross-kingdom RNAi has evolved in other classes of plant pathogens as well.

Oomycetes comprise some of the most destructive plant and animal diseases, and belong to the eukaryotic lineage of the Stramenopiles, that is phylogenetically far distant from animal, plants, and fungi. To clarify the existence of cross-kingdom RNAi in plant-oomycete interactions, we investigate the infection of *Arabidopsis thaliana* by the oomycete pathogen *Hyaloperonospora arabidopsidis* causing the downy mildew disease. We here show that *A. thaliana* mutants compromised in the microRNA pathway, including the *argonaute* (*ago*)*1-27,* resisted infection by *H. arabidopsidis* suggesting a negative role of AGO1 in defense against this pathogen. Purifying *At*AGO1 from infected *A. thaliana* coupled with sRNA next generation sequencing analysis revealed >100 oomycete sRNAs that translocated into the host plant. Remarkably, some plant genes predicted to be targets of these oomycete sRNAs, including two members of the *A. thaliana With No Lysine(K)* (*AtWNK*) *Kinase* family, *AtWNK2* and *AtWNK5*, as well as the extracellular protease, *AtAED3*, were suppressed during infection. Moreover, T-DNA insertion lines of these targets, including *atwnk2-2*, *atwnk2-3, atwnk5-3,* and *ataed3-1*, all exhibited enhanced susceptibility to *H. arabidopsidis* infection, demonstrating that host targets of such invasive oomycete small RNAs are important for pathogen defense.

We here provide evidence that cross-kingdom RNAi has evolved in plant-oomycete interaction and thus seems to be a widespread virulence mechanism among diverse microbial pathogens.

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[P48] Molecular assessment of metabolome alterations in carrots induced by abiotic stress challanges

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Besides its unrivalled colour, fresh cultivated carrots (*Daucus carota* L.) and its products are favored by the consumer for its typical sweet flavor. Unfortunately, the attractive sensory quality of carrots is hindered by a sporadic bitter off-taste which is often the reason for consumer complaints and therefore a major problem for vegetable processors. This off-flavor is induced by abiotic and biotic stress factors during harvesting, transportation, storage and processing (1-3). Although recent application of a Sensomics approach gave first insight into individual bitter tasting phytochemicals in carrots (4), their up-regulation during abiotic stress still remains elusive.

In order to gain a more comprehensive knowledge on the chemical mechanisms involved in taste changes of cultivated carrots in response to abiotic stress factors, like water stress, different stressed and non-stressed carrot genotypes were comparatively screened by application of a fast and robust high-throughput UPLC-TOF-MS metabolic profiling analysis. Software-assisted marker molecule selection in stressed carrots, followed by preparative chromatographic purification revealed the chemical structures of bitter tasting phytochemicals by means of LC-MS, LC-MS/MS, and 1D/2D-NMR experiments. Accurate quantitation of these target molecules by means of UPLC-MS/MS_{MRM}-ECHO techniques in carrots before and after abiotic stress challenge of the same genotypes revealed for the first time novel insights into the stress-induced metabolic response of distinct carrot genotypes. These results might help to navigate carrot breeding programs and to optimize post-harvest treatment of carrots from producer to consumer/processor towards the production of high quality carrot products.

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[P49] Salt Sequestration in Quinoa

Messerer, M., Böhm, J., Müller, H. M, Scherzer, S., Maierhofer, T., Ache, P., Shabala, S., Haberer, G., Zhang, H., Zhu, J-K., Hedrich, R., Mayer, K.F.X.

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Soil salinity is one of the major environmental factors causing crop loss worldwide. The facultative halophyte *Chenopodium quinoa* is a highly nutrious crop which is related to sugar beet and spinach. For scientists and crop breeders, its salt tolerance is a characteristic of special interest. Quinoa is able to grow when watered with seawater and to sequester salt to so called epidermal bladder cells (EBCs). Upon removal of salt bladders, quinoa becomes salt-sensitive. Despite the agronomic importance of quinoa, the molecular mechanism underlying the unique salt dumping capabilities of bladder cells in this species is not understood.

We have analyzed the salinity-induced changes in the EBC transcriptome by contrasting RNAseq data of EBCs with leaf cells under control and salt conditions. The analysis of differentially expressed genes (DEGs) between the two cell types revealed their distinct roles in contributing to salt tolerance. Based on these results we could further investigate salt-dependent shifts in transcriptomic and metabolomic pathways using the tool PaintOmics (1). Next, we identified quinoa specific compatible osmolytes – so called osmoprotectans which balance the osmotic pressure of the increasing salt load – and the corresponding transport system. A general overview of the results with a special focus on transcriptomic pathway analysis will be presented.

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[P50] A Comprehensive Survey of Pseudogenes and Gene Fragments in Barley

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Pseudogenes are gene-like sequences featuring serious mutations which destroy their original function. Although originally dismissed as “junk DNA”, they are now increasingly studied in mammals, due to mounting reports of pseudogene functionality. Studies in more complex plant genomes such as the *Triticeae* were hampered by the absence of reference genome sequences. The barley genome (5 Gb) is the first average sized plant genome assembled to an unprecedented completeness, and was also among the first to be included in our detailed plant pseudogene survey.

Using a homology-based approach and a query set of 38,157 high confidence barley genes, we identified 11,015 full length pseudogenes and 78,425 smaller pseudogene fragments. Interestingly, the common intron-based classification of pseudogenes into ‘duplicated’ and ‘retroposed’ demonstrates a surprisingly small contribution of retrotransposon mediated sources. A major proportion of pseudogenes originates from tandem cluster situations or is located in close vicinity to their parent gene. The overall chromosomal pseudogene distribution follows that of genes, with high densities towards the telomeres. Gene family relationships, functional biases and transcriptional evidences were investigated. For example, we found transcriptional evidence for 7,018 pseudogenes - including 1,340 full length pseudogenes. They hold the potential to take part in regulatory processes.

We investigated syntenic regions of cultivated barley and four wild barley variants: Two Tibetan wild barley variants and two wild variants growing closely together on opposing slopes of the ‘Evolution Canyon’ I in Israel. Being affected by drastically different microclimates, they present the opportunity to study pseudogene evolution in closely related plants and in comparison to a domesticated relative. We present selected examples of very recent pseudogenization events between cultivated and wild barley varieties.

[P51] The WHEALBI Panel: Unveiling genomic asymmetries in the bread wheat genome.

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The diversity encompassed by the worldwide panel of 488 wheat accessions provides a comprehensive insight into the ancestry, domestication, improvement and breeding history of wheat. The panel comprises samples representing different levels of ploidy, cultivation states (wild, landrace, cultivar, breeding) as well spring and winter wheat which provides the foundation for analyses of diversity on a multidimensional scale. The variation data, derived from the extensive exome capture sequencing efforts in this project and the newly completed wheat reference sequence (Chinese Spring, CS), provides the most comprehensive resource available for wheat research to date. A survey of selection signatures, asymmetries in genetic plasticity and phylogenetic relationships of this resource on a subgenome, ploidy, trait, temporal and spatial level unveils clear distinction of wheat material based on vernalization requirement, cultivation state and geographical origin. Phylogenetic analysis of the diversity in the panel is in close agreement with the wheat ancestry described by Kilian et al. and provides additional support for the proposed origin of the D genome. The variation data also favors two major expansion routes starting from a single domestication site in the Fertile Crescent. An additional major benefit of the Whealbi project are large scale field trials on different geographical locations monitoring various agronomically important traits including disease resistance, drought tolerance and yield. The combined analysis of these phenotypic and genetic traits by GWAS reveals, besides known improvement and domestication loci, also novel candidates for flowering time and plant height.

[P52] The genomes of western civilisation

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Bread wheat (*Triticum aestivum*) has strongly shaped human civilization since its domestication at the Neolithic revolution. Today, wheat is one of the top three crops in agriculture and provides ~20% of all calories of the world's population. Another cereal of major importance for brewing and animal feed is barley (*Hordeum vulgare*). Whole genome sequencing projects have shown to provide invaluable tool boxes both for academic research as well as for agricultural and applied sciences for many plant species before. In contrast to two other major crops – maize and rice, whole genome projects have been impeded by several notoriously difficult properties of both the wheat and barley genome. With 17 Gigabases in size, the hexaploid wheat genome (three subgenomes) is more than five times larger than the human genome and the high repeat content of cereal genomes complicates whole genome assembly from short sequence reads.

While earlier whole genome sequencing attempts for wheat (1,2) and barley (3) mainly resolved the gene space and allowed first insights into the genome organization and structure (4), recent technology improvements and novel strategies brought a breakthrough in cereal genomics. HiC chromosome capture as well as novel assembly algorithms allowed the generation of both a barley and wild emmer wheat reference genome sequence recently (5, 6), enabling access to 39,000 high-confidence barley gene models ordered on highly contiguous pseudochromosomes. In the framework of the IWGSC (International Wheat Genome Sequencing Consortium) (7), a highly improved genome assembly of bread wheat has been generated using a large array of technologies. These new resources will greatly assist targeted breeding and contribute to food security in a changing environment.

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[P53] Mapping the Arabidopsis Proteome

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Three decades after its promotion as the model organism of choice for plant biology research, Arabidopsis, as the most thoroughly studied species of flowering plants, still remains the standard reference for many aspects of plant science. In addition to the already existing transcriptomic expression profiles, a proteomic analysis of Arabidopsis tissues can provide further biological information about protein localizations and abundancies, as well as post-translational modifications that is not accessible based on transcriptomics data alone. Here we present a quantitative proteome map for *Arabidopsis thaliana* based on the in-depth proteomic profiling of 30 different tissues, developmental stages and cultured cells. Tissues were measured to a depth of 14500 +/- 900 proteins per tissues, almost on par with our corresponding mRNA profiling of 17000 +/-2800 transcripts per tissue. Overall, we found evidence for 89 % of the annotated protein-coding genes on transcriptome level and 66 % on protein level. Almost half of the identified proteins can also occur in a phosphorylated state, as shown by our phosphoproteomic analysis, which in total resulted in the identification of 36000 high confidence phosphorylation sites. Our analysis of the core proteome of around 8000 proteins, expressed in all the tissues that were included in this study, further showed, that not qualitative but quantitative protein composition seems to characterize and distinguish specific tissues. Indeed, only very few proteins exhibited a truly tissue-specific expression pattern. By using our dataset to construct co-expression networks based on transcriptomic and proteomic data we hope to elucidate, what additional information can be gained from the protein level information, when assessing the functional role and correlation of individual genes/proteins at a system-wide scale. Taken together, this study will provide a refined transcriptomic, proteomic and phosphoproteomic tissue-resolved baseline for future research in Arabidopsis.

[P54] Novel insights into the ligand-receptor pair SCFE1/PCFE1 and RLP30

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Arabidopsis thaliana has evolved a large number of transmembrane cell surface receptors that play major roles during development and are important to respond to external stimuli. We have associated one *Arabidopsis* leucine-rich repeat (LRR) receptor-like protein, RLP30, with a specific function as pattern recognition receptor for the elicitor SCFE1 (Sclerotinia culture filtrate elicitor 1)[1]. RLP30 lacks an intracellular kinase domain and therefore associates with the adaptor kinase SOBIR1 for signaling, and additionally recruits the kinase BAK1 after ligand binding[2]. SCFE1-insensitive *Arabidopsis* accessions have revealed important regions in the RLP30 receptor, which are most likely implicated in ligand binding or interaction with SOBIR1.

SCFE1 promotes typical MAMP (microbe associated molecular pattern) - induced defense responses in *Arabidopsis*, similarly to the novel elicitor PCFE1 (Pseudomonas culture filtrate elicitor 1) from different *Pseudomonas* strains. Biochemical analyses revealed that both elicitors share the same properties and even more strikingly, are both recognized by RLP30. We believe that SCFE1 and PCFE1 share at least the same minimal binding motif, but more likely, the elicitor activity is derived from homologous proteins. So far, this is one of the very few examples of an elicitor that occurs across different kingdoms.

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[P55] Should I burst or should I grow – The protein phosphatases ATUNIS1/2 are negative regulators of pollen tube cell wall integrity.

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Plant reproduction is a fierce competition. Pollen tubes (PTs) must grow as fast as possible in order to contribute to the next generation, as the number of receptive ovules is limited. Thus, growing PTs have to closely monitor their cell wall integrity (CWI) and coordinate it with their internal growth machineries. If the cell wall becomes too rigid, PT growth is stalled. If the cell wall is too elastic, the PT might burst precociously and thus not reach the ovules. As a result, the yield will be decreased.

In the model plant of genetics, *Arabidopsis thaliana*, ANXUR1/2 (ANX1/2) govern the pollen tube CWI pathway. They are two redundant receptor-like kinases of the *Catharantus roseus* receptor-like kinase 1-like (*Cr*RLK1L) family. *anx1 anx2* plants are male sterile. *In vitro, anx1 anx2* pollen grains are unable to form PTs and burst after forming little bulges [1,4]. *ANX1/2* over-expressor lines form PTs that are eventually inhibited in growth due to cell wall material over-accumulations at the PT tip [2]. Consequently, the plasma membrane invaginates at the PT tip. Till date, three other genes are known to act in the ANXUR-dependent CWI pathway: two partially redundant membrane-localized NADPH oxidases (RbohH/J) and a receptor-like cytoplasmic kinase named MARIS (MRI)[3]. All of them are positive regulators of CWI.

Here, we report the discovery of two novel regulators of the ANX-dependent CWI pathway, protein phosphatases ATUNIS1/2 (AUN1/2). The pollen-preferentially expressed genes are 89.8% identical at the amino acid level. An EMS mutant screen revealed a suppressor mutant of the *anx1 anx2* pollen bursting phenotype. The mutation triggers a non-synonymous D94N substitution in the core catalytic domain of AUN1. *AUN1* and *AUN1*$^{[D94N]}$ were cloned, fused with YFP and introduced into WT-Col0, *anx1 anx2*, *artificial micro-RNA of ligand of ANXUR (amiRNAloa)*, *rbohH rbohJ* and *mri/+* plants. I could show that AUN1/2 are negative regulators of CWI and that *AUN1*$^{[D94N]}$ rescues the pollen bursting phenotype of *anx1 anx2, amiRNAloa* and *rbohH rbohJ* plants. Also, I demonstrate that AUN1$^{[D94N]}$ is a dominant negative form of AUN1.

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[P56] Blueberry Yield Instability is Caused by Photosynthetic Limitation Under UK Climatic Conditions

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Under UK growing conditions, blueberry growers experience large annual variations in crop yield however almost nothing is known regarding the underlying cause of this significant economic problem. In order to address this lack of knowledge, we tested the hypotheses that i) yield instability is caused by catastrophic environmental events leading to severe developmental interruption or that ii) subtle environmental variations results in year-to-year yield variation.

Analysis of yield potential throughout the growing season revealed that yield loss occurred throughout fruit development and was not associated with specific environmental events. Physiological data generated from light-response and A-C_i curves indicated that photosynthesis (A) was saturated at relatively low light irradiance (~500 μmol m^{-2} s^{-1}) and is restricted by the carboxylation efficiency of RuBisCO at ambient CO_2 levels. Concomitant gas exchange and chlorophyll fluorescence analysis of blueberry leaves at different temperatures indicated a reduction of A at low temperatures mainly due to reduced electron transport rate. Furthermore the response of photosynthesis and stomatal conductance (g_s) to changing irradiance was asynchronous, resulting in noncoordination between A and g_s in dynamic light environments. Quantification of sugar and starch dynamics in plant organs indicated that fruit growth depends primarily on the daily production of carbohydrates by leaves and there is no accumulation of a starch buffer to allow continued ripening under conditions limiting for photosynthesis. Monitoring of the growing environment indicated significant fluctuations in temperature and light throughout the growing season in the maritime environment of the UK.

We conclude that a significant component of yield variability results from variation in light irradiance as well as temperature in different growing seasons. In particular, the crop is unable compensate for days of low irradience by enhanced photosynthesis under days of high irradience. This work indicates the need to develop blueberry varieties more suited to yield under UK growing condtions.

[P57] A sub-compatible *Rhizobium* strain exploits an intercellular infection mechanism to colonize *Lotus*

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Intercellular infection has been postulated as an ancient infection mode that precedes the evolutionary innovation that is infection thread formation (1). Although *Lotus* becomes infected through an infection thread dependent mechanism by its compatible symbiont *Mesorhizobium loti (Ml)*, there is evidence that it maintains a genetic program for intercellular infection and cortical bacterial uptake into single cells from the apoplast (1).

The *Rhizobium leguminosarum* strain Norway (*Rl* Norway) was isolated from *L. corniculatus* nodules, although *R. leguminosarum* strains belong to a different cross-inoculation group. Moreover, *Rl* Norway induces ineffective nodules in different *Lotus* species and ecotypes (2). This suggests that the interaction between *Lotus* and this strain is sub-compatible. Here we postulate that this sub-compatible strain uses an intercellular infection pathway to colonize *Lotus* and probably enters cortical cells directly from the apoplast.

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[P58] Chromosome-level assembly of *Arabidopsis thaliana* Niederzenz-1

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The majority of genomic research on *Arabidopsis thaliana* is based on the Columbia-0 (Col-0) reference genome sequence. Reference-based variant analysis was performed for many years manly focused on small nucleotide polymorphisms.

In order to enable analyses of variation larger than few nucleotides in length, we assembled the Niederzenz-1 (Nd-1) genome sequence completely *de novo* based on second generation sequencing reads and performed *ab initio* gene calling (1). Incorporation of exon hints von Col-0 into the gene calling procedure enabled the prediction of genes with non-canonical splice sites (2). Comparisons between Col-0 and Nd-1 confirmed the overall synteny of both genomes (1) by a high number of matches between both gene sets. In a next step, we applied single molecule real time (SMRT) sequencing to generate an even more continuous Nd-1 genome sequence. In this assembly chromosome arms are almost completely represented by a single contig (N50 = 9.3 Mbp), while smaller contigs cover pericentromeric sequences. The total nucleome assembly of 119.5 Mbp matches the size of the Col-0 reference sequence. A genetic map was constructed to orientate and position the contigs resulting in pseudochromosomes. Exon-hints from Araport11 were incorporated into gene calling to improve gene annotation quality. In addition, the identification of large structural variants up to 1 Mbp as well as investigations of gaps in the Col-0 reference sequence were enabled. Experimental validation of some structural variants by PCR and Sanger sequencing was used to validate the correctness of the assembly. This assembly contributes to the *Arabidopsis thaliana* pan-genome and facilitates comparative genomics as well as investigations of transposable elements.

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[P59] Understanding lateral root formation in Arabidopsis

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Intercellular communication is central for the development of an organism. It is mediated by biochemical gradients as well as physical forces that collectively regulate differentiation and development. Lateral root formation is a developmental process in which the integration of chemical signals and physical forces is evident. During its development, the lateral root heavily depends on spatial accommodating responses of overlaying cell layers. We have shown that pericycle cells need to swell in order to undergo formative divisions, whereas the overlying endodermis undergoes a dramatic volume loss during lateral root formation. Through manipulating SHY2-mediated auxin signaling in the endodermis, we were able to completely block cell proliferation in the pericycle. It appears that the pericycle perceives this non-accommodating endodermis as an increased resistance to its expansion growth. The pericycle-endodermis interaction now provides a unique opportunity to elucidate the molecular and cellular mechanisms underlying the interplay between cell volume regulation and mechanosensing during plant development. To that end, we have designed a forward genetic screen to identify suppressors of impaired accommodation response phenotype. We also aim at understanding the role of auxin transport in spatial accommodating responses.

[P60] Stem-cell niche formation in flowers requires combined activity of REVOLUTA and LEAFY target REGULATOR OF AXILLARY MERISTEMS1

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Flower morphogenesis requires a transient meristematic state. In *Arabidopsis* this is characterized by the transient expression of the stem-cell niche marker *WUSCHEL* (WUS) at the centre of floral buds from stage 2 to stage 6. It has been proposed recently that *WUS* expression in young flower primordia could be induced by a combination of signals diffusing from the L1. However how WUS early expression is linked to flower founder-cell specification remains elusive. In this work we show that two redundant genetic pathway, known to be involved in flower formation, also control flower meristem initiation. The transcription factors LEAFY (LFY) and REVOLUTA (REV) converge towards stem-cell niche formation in flowers. LFY acts through regulation of the transcription factor REGULATOR OF AXILLARY MERISTEMS1 (RAX1), which is also involved in axillary meristem initiation at leaf axils. Furthermore, when both pathways are disrupted as in *rax1 rev* double mutant *WUS* expression is reduced and mislocated, compromising stem-cell niche initiation and therefore flower formation. Putative targets of RAX1 potentially involved in meristem homeostasis were investigated.

[P61] Functional conservation of RLK-mediated cell wall integrity signaling in tip-growing cells throughout land plants.

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Plant cells are in a constant need to sense their environment to facilitate fundamental biological processes such as cell growth. Especially tip-growing cells such as pollen tubes (PTs) and root hairs (RHs) rely on an immaculate interplay between vacuolar delivery of cell wall material and turgor-driven, unidirectional cell expansion. During the last decade major advances have been made in identifying Malctin-like receptors (MLRs) of the Receptor-like kinase (RLK) family as key-regulators of CWI signaling in *Arabidopsis thaliana*. MLRs have been shown to influence crucial agronomical traits such as plant architecture, fertility, crop yield and immunity in a variety of flowering plants. The *Arabidopsis* RLKs ANXUR1/2 (ANX1/2) and FERONIA (FER) belong to the Malectin-like domain(MLD)-containing subfamily of *Catharanthus roseus* RLK-1-like proteins (*Cr*RLK1Ls). ANX1/2 and FER were shown to regulate CWI in pollen tubes and root hairs, respectively. Interestingly, downstream signaling in both PTs and RHs depends on the cytoplasmic RLK MARIS (MRI) and paralogs of the same class of NADPH oxidases - the RESPIRATORY BURST OXIDASE HOMOLOGUES H, -J and -C (RBOH-H, -J and -C), supposedly in a Ca^{2+}-dependent manner.

1) The remarkable phenotypic identity and genetic relationship between mutant lineages of these CWI-regulatory genes and Ca^{2+} channel mutants of the CYCLIC-NUCLEOTIDE-GATED-ION-CHANNEL (CNGC) class will be presented. May CNGCs be the missing downstream link between RLK-mediated and Ca^{2+}-dependant CWI signaling in both, pollen tubes and root hairs?

2) A major phenotypic screen in the liverwort *Marchantia polymorpha* revealed the *Cr*RLK1L-homolog *Mp*THE and the MRI-homolog *Mp*PTI as putative CWI regulators in rhizoids which expand by tip-growth as well. Follow-up experiments will be presented. May RLK-mediated signaling have been conserved not only between cell types, but throughout land plant evolution?

Such functionally conserved CWI signaling modules under control of MLRs may represent promising tools for agricultural and biotechnological optimizations.

[P62] Root fungal endophytes: what did we learn and what is there to do for strengthening plant growth and yield?

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Climate change and an exponential growth of the world population demand an increase of arable farmland and conservation of natural resources including soil. An ecological agriculture with soil microorganisms as key players is required, especially considering drought- and temperature-related stresses. Research in plant-soil-microbe interactions in the rhizosphere has shown that plants as holobionts build microbial communities and mutualistic associations regulated by their root exudates to get benefits for plant growth, health and fitness (1). Well-studied root mutualistic associations include root-nodulating bacteria, plant growth promoting microorganisms and mycorrhizal fungi, but non-mycorrhizal endophytic fungi are often neglected in most of the plant-microbe interaction studies. Thus, little is known about their benefits, ecology and functions in plant rhizospheres though they are broadly represented by members of the phyla Basidiomycota, Ascomycota and Mucoromycotina and are also important factors in the plant microbiome (2, 3, 4). We have characterized newly isolated root fungal endophytes from tomato crops together with the model endophyte S*erendipita indica*. They improve plant growth, yield and tolerance towards abiotic stress and can solubilize phosphate and protect plants against pathogens (5, 6, 7). Now our challenge is to better understand how root endophytic fungal communities are assembled and regulated by crop plants, which plant and fungal molecular mechanisms are needed for growth promotion and tolerance to stress and what fungal compounds and signals are required in microbe-microbe interactions. With this knowledge, we will be able to ecologically engineer microbiomes and reduce environmental impacts of the current plant production systems without reducing soil quality, plant yield and yield quality.

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