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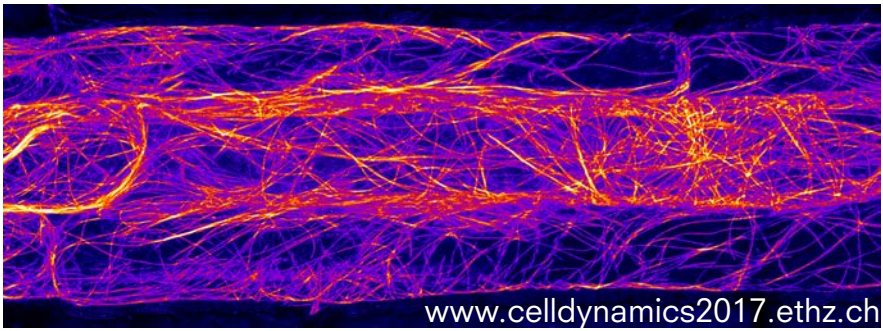
Zurich-Basel Plant Science Center

PSC SYMPOSIUM

30 November & 1 December 2017, ETH Zurich



Dynamics of Plant Development & Evolution

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30 November & 1 December 2017, ETH Zurich

Dynamics of Plant Development & Evolution

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Content

Organization

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The competence center for plant science research at ETH Zurich,

University of Zurich and University of Basel.

→ **www.plantsciences.ch**

Venue

ETH Zurich

Auditorium Maximum (HG F30), Rämistrasse 101

Symposium website

→ **www.celldynamics2017.ethz.ch**

Admission

PSC members: free of charge.

Other participants: CHF 50.

Program

Thursday, 30 November

8.45 Welcome

Selection & adaptation

9.00 **Chris Morgan**, John Innes Center, UK
9.30 **Cris Kuhlemeier**, U Bern, CH
10.00 **Juliette de Meaux**, U Cologne, DE

10.30 Coffee break with poster session

Response to signals

11.00 **Fredy Barneche**, IBENS Paris, F
11.30 **Daniel Croll**, U Neuchâtel, CH
12.00 **Ueli Grossniklaus**, U Zurich, CH
12.30 **Claudia Köhler**, SLU Uppsala, SE

13.00 Lunch break with poster session

Communication

14.30 **Marja Timmermans**, U Tübingen, DE
15.00 **Thomas Greb**, COS Heidelberg, DE
15.30 **Ross Sozzani**, North Carolina State U, USA
16.00 **Monika Hilker**, FU Berlin, DE

16.30 Poster awards
16.45 Apéro and poster session

Friday, 1 December

Morphogenesis

8.30 **Olivier Hamant**, ENS Lyon, F
9.00 **Karin Schumacher**, COS Heidelberg, DE
9.30 **Elizabeth Haswell**, Washington U in St. Louis, MO, USA

10.00 *Coffee break*

10.30 **Elena Kramer**, Harvard U, USA

Polarity & (a)symmetries

11.00 **Dolf Weijers**, U Wageningen, NL
11.30 **Ralf Reski**, U Freiburg, DE
12.00 **Dominique Bergmann**, U Stanford, USA
12.30 **Erik Nielsen**, U Michigan, USA

13.00 Young scientists and speakers lunch discussion (reservation necessary).

Invited speakers

in speaking order

Chris Morgan

John Innes Centre, Norwich, UK

The evolution of meiosis in *Arabidopsis arenosa*

Meiosis is essential for fertility of sexual eukaryotes and its core structures and progression are conserved across kingdoms. Nevertheless, meiosis is challenged by changes such as genome duplication and environmental stresses. How can such a conserved system evolve in response? In a genome scan for adaptation to whole genome duplication in *Arabidopsis arenosa*, we found evidence that eight interacting meiotic proteins were under strong selection during the evolution of the polyploid lineage. The proteins encoded by these genes are critical for axis formation, recombination and synapsis. We hypothesize that modifications of these proteins are important for stabilizing polyploid meiosis, and that they function by reducing crossover rates and preventing multivalent associations among the available chromosome copies. We also found that two of the same genes show strong evidence of having independently been under selection in a diploid lineage that colonized a warmer lowland habitat. That distinct alleles of the same genes were under selection after both whole genome duplication and habitat colonization hints that modifications of core axis components may be critical in different evolutionary scenarios. Our findings are broadly relevant to understanding the evolutionary dynamics of proteins that participate in large constrained complexes.

Cris Kuhlemeier

University of Bern, CH

The molecular basis of pollinator-mediated speciation in *Petunia*

The genus *Petunia* (Solanaceae) comprises species that are pollinated by bees, nocturnal hawkmoths or hummingbirds. Rapid adaptation to new pollinators has happened frequently in many different taxonomic groups. For instance, in the Solanaceae the evolution of hummingbird pollination has been documented at least ten times. Shifts in pollinator preferences can lead to reproductive isolation and ultimately speciation. Such shifts require the modification of multiple floral traits, among them visible color, UV absorption, scent, nectar as well as morphology. How can such complex changes happen again and again over short periods of evolutionary time? To elucidate the molecular basis of pollinator-driven adaptation and speciation in Petunia we use a combination of genetics, genomics, biophysics and behavioral ecology. Our data indicate that shifts in pollination syndromes are caused by a limited number of mutations of large phenotypic effect. In fact, even the modification of single genes can strongly affect pollinator preference and thereby cause reproductive isolation. Knowledge of the causative genes gives insight into the process of speciation and helps to resolve phylogenetic relationships in recent radiations.

Juliette de Meaux

University of Cologne, DE

Does defense coevolve with flowering time in *A. thaliana?*

The selective impact of pathogen epidemics on host defenses can be strong but remains transient. By contrast, life-history shifts can durably and continuously modify the balance between costs and benefits of immunity, which arbitrates the evolution of host defenses. Their impact, however, has seldom been documented. Here, we show with a simple mathematical model how the optimal investment into defense is expected to increase with increasing lifespan. We further document that in natural populations of the model plant *Arabidopsis thaliana*, the expression levels of defense genes correlate positively with flowering time, which in annual species is a proxy for lifespan. Using a novel genetic strategy based on bulk-segregants, we partitioned lifespan-dependent from lifespan–independent defense genes and could demonstrate that this positive co-variation can be genetically separated. It is therefore not explained by the pleiotropic action of some major regulatory genes controlling both defense and lifespan. Moreover, we find that defense genes containing variants reported to impact fitness in natural field conditions are among the genes whose expression co-varies most strongly with flowering time. In agreement with our model, this study reveals that natural selection has likely assorted alleles promoting higher expression of defense genes with alleles that increase the duration of vegetative lifespan in A. thaliana and vice versa. This is the first study documenting the pervasive impact of life history variation on the maintenance of diversity in host immunity within species.

Fredy Barneche

IBENS, Paris, FR

Light signaling controls nuclear architecture reorganization during seedling development

Nuclear and chromatin organization undergo large rearrangements along plant life in response to endogenous and environmental signals. Phenotypic changes of the nucleus are typically orchestrated during cell specialization linked to developmental adaptations, as observed during embryonic and post-embryonic development, but their functional significance is poorly understood. For example, both the size and heterochromatin patterns are subject to major variations during cotyledon development, ultimately forming conspicuous sub-nuclear foci referred to as 'chromocenters' in adult seedlings. Chromocenter establishment is known to rely on the coordinated condensation of heterochromatic pericentromeric regions and transposable elements around the centromeres, consequently influencing the higher-order organization of gene-rich euchromatic domains in the nuclear space. We observed that chromocenter formation during cotyledon development requires light perception and is dependent on specific photoreceptors and downstream signaling integrators DET1/COP1 independently from nuclear size variations. Cotyledon photomorphogenesis further leads to a strong reduction in chromatin mobility and a 3-fold increase in RNA Polymerase II activity, suggesting a transition from globally quiescent nuclei in etiolated cotyledons to a more active transcriptional status in photoautotrophic cotyledons. We also observed that under certain light conditions such as darkness, expression of the linker histone H1.3 variant triggers heterochromatin re-organization, possibly by competing with more canonical histone H1 isoforms for common loci to influence the epigenome activity. Hence, our data indicate that light-dependent heterochromatin dynamics largely result from a tight control of histone H1.3 protein abundance. These findings unveil how a specific environmental signal is translated in a molecular determinant of chromatin status to control nuclear organization in a plant system.

Daniel Croll

University of Neuchâtel, CH

How pathogens rapidly surmount plant resistance in agricultural ecosystems

Pathogens play a central role in the ecology and evolution of plants. In agricultural ecosystems, microbial pathogens show enormous evolutionary potential to surmount disease resistance of hosts and adapt to changes in the environment. However, the genetic basis of rapid pathogen adaptation is poorly understood. We analyzed genomes of fungal pathogen populations and combined this with association mapping to retrace the processes of recent adaptive evolution. Adaptation to overcome host resistance was largely mediated by mutations in effector gene loci in highly polymorphic regions of the pathogen genome. The pathogen evolved distinct mechanisms to infect different host genotypes and the genetic basis varied from single locus to highly polygenic adaptations. We found that the emergence of fungicide resistance evolved from a polygenic basis including functions in stress response pathways and modifications of the fungicide target. To retrace the evolutionary origin of key pathogen innovations, we assembled complete genomes of multiple strains. We found that genomes of the same pathogen species showed substantial structural variation. Recent gene gains and losses led to rapid evolution of gene content in populations and contributed to gains in pathogenicity. Chromosomal rearrangements and the maintenance of variation are likely dominant drivers of pathogen adaptation.

Ueli Grossniklaus

University of Zurich, CH

Receptor-like kinase-mediated signalling during plant reproduction

Research in our laboratory focuses on the developmental genetics of plant reproduction, with an emphasis on cell-cell communication during double fertilization. Fertilization depends on the proper reception of the pollen tube by the synergid cells, where the pollen tube arrests growth and ruptures to release the sperm cells. We have shown that receptor-like kinases (RLKs) of the *Cr*RLK1L subfamily play an important role in reproduction, with the FERONIA RLK acting in the female and the two redundant ANXUR (ANX1/2) RLKs in the male gametophyte (Escobar-Restrepo et al. *Science* 317: 656; Boisson-Dernier et al. *Development* 136: 3279; Miyazaki et al. *Curr Biol* 19: 1327). The *Arabidopsis* genome encodes 17 plant-specific *Cr*RLK1L family members with a multitude of functions in pollen tube reception, pollen tube growth but also phytohormone signalling, mechanosensing and plant defense. The extracellular domain of *Cr*RLK1Ls has similarities with malectin (Boisson-Dernier et al. *J Exp Bot* 62: 1581), suggesting that it may bind carbohydrate moieties, but it was also reported to bind the small, secreted RALF peptide (Haruta et al. *Science* 343: 408). Thus, the functions of *Cr*RLK1Ls are diverse and complex. Using various forward and reverse genetic approaches, we are in the process of identifying additional components in the *Cr*RLK1L signalling cascade, focusing on both upstream and downstream components of this RLK subfamily. I will report on the characterization of new components that regulate pollen tube integrity and growth through the *ANX* pathway.

Claudia Köhler

SLU, Uppsala, SE

Epigenetic regulation of transposable elements drives plant speciation

Polyploidization is a widespread phenomenon among plants and is considered a major speciation mechanism. Polyploid plants have a high degree of immediate post-zygotic reproductive isolation from their progenitors, as backcrossing to either parent will produce mainly nonviable progeny. This reproductive barrier is called triploid block and it is caused by malfunction of the endosperm. Our work revealed that deregulated parent-of-origin specific imprinted genes are causal for the response to interploidy hybridizations, revealing an epigenetic basis of this phenomenon. Imprinted gene expression is a consequence of silencing mechanisms targeting transposable elements (TEs), establishing TEs as main drivers of plant speciation. Interestingly, imprinted genes establishing hybridization barriers act themselves as regulators of heterochromatin formation, revealing striking parallels to hybrid dysgenesis in *Drosophila* that has also been linked to heterochromatic repeats. I will discuss the mechanisms establishing interploidy hybridization barriers in plants, highlighting the role of TEs and their regulation by small RNAs in this process. Furthermore, I will show that similar mechanisms act to establish interspecies hybridization barriers in the genus Capsella, where number of paternally-expressed imprinted genes correlate with the strength of hybridization barriers between species. Our data suggest that variation in TE number and the resulting differences in paternally-expressed imprinted genes underlie the effective ploidy variation between species of different breeding system histories, and as a consequence, cause the establishment of endosperm-based hybridization barriers.

Marja Timmermans

University of Tübingen, DE

Small RNAs as mobile, morphogen-like signals in development

Adaxial-abaxial (top-bottom) polarity drives the flattened outgrowth and patterning of leaves, and represents an important innovation in the evolution of land plants. Patterning of this axis is driven by an intricate gene regulatory network. Integral to this network are two sets of conserved transcription factors that promote either adaxial or abaxial fate, and are expressed in complementary domains on the top or bottom side of the leaf, respectively. The positional information needed to delineate these domains is provided in part by the small RNAs miR166 and tasiR-ARF. We have shown that these small RNAs move outside their defined domain of biogenesis and form opposing gradients across the leaf that polarize expression of key adaxial- and abaxial-promoting transcription factors, HD-ZIPIII and ARF3/4, respectively. Our observations, which will be presented, indicate that mobile small RNAs have morphogen-like activity and generate sharply defined domains of target gene expression through an intrinsic and direct threshold-based readout of their mobility gradients. This readout is highly sensitive to small RNA levels at the source, allowing plasticity in the positioning of a target gene expression boundary. Besides patterning their immediate targets, the readouts of opposing small RNA gradients enable specification of robust, uniformly positioned developmental boundaries. Mobile small RNAs and their targets thus emerge as highly portable regulatory modules through which to create pattern; providing a compelling basis for the extensive conservation and repeated co-option of developmentally important small RNA-target modules.

Thomas Greb

COS Heidelberg, DE

Cell fate decisions during radial plant growth – making a case for phloem specification

Body shaping in multicellular organisms depends on the activity of distinct stem cell niches coordinated over long distances. Radial growth of plant shoots and roots is based on the tissue-forming properties of a group of stem cells called the cambium, the activity of which leads to the production of water and sugar transporting tissues (xylem and phloem). Considering its function as a stem cell niche that is essential for the constant production of vascular tissues, the cambium represents an ideal model for addressing questions concerning the regulation of cell identity and how growth processes are aligned with endogenous and exogenous requirements. Here, we show that SMXL genes, associated with the signaling of the plant hormone strigolactone (SL), are expressed in developing phloem cells along the entire plant body. We demonstrate that, within the SMXL gene family, specifically SMXL3/4/5 deficiency results in strong and general defects in phloem formation, altered sugar accumulation, and seedling lethality. By comparing protein stabilities, we also show that SMXL3/4/5 proteins function differently to canonical SL signaling mediators, although being functionally interchangeable with those under low SL signaling conditions. Our observations reveal a fundamental mechanism of phloem formation and indicate that diversity of SMXL protein functions is essential for a steady fuelling of plant meristems. By analyzing the functional relevance of distinct protein-protein interactions, the molecular action mode of the nuclear-localized SMXL proteins is beginning to emerge.

Ross Sozzani

North Carolina State U, USA

Modeling gene regulatory networks that control the *Arabidopsis* root stem cells

The stem cells in the tip of the *Arabidopsis* root form all the root tissues by undergoing rounds of coordinated cell division while maintaining their undifferentiated state. A better understanding of the transcription factors that maintain the stem cells and control each stem cell's identity would give us more insight into how the growth and development of the root is initiated. While a number of transcription factors involved in root stem cell maintenance have been described, a comprehensive view of the transcriptional signature of the stem cells is lacking. We have generated a model of the transcriptional mechanisms underlying the identity and maintenance of the *Arabidopsis* root stem cells that links known and newly predicted factors involved in these processes. This model led to a map of genetic interactions that orchestrate the transcriptional regulation of stem cells.

Monika Hilker

FU Berlin, DE

Insect egg deposition warns plants of impending larval herbivory

Plants are able to perceive egg depositions by herbivorous insects and respond by defenses targeting the eggs or the hatching larvae. How do plants "recognize" the egg depositions on their leaves? Several compounds of amphiphilic character have been identified that are released with the eggs and elicit defensive plant responses. The egg-induced plant defenses include (i) formation of necrotic tissue and neoplasms which result in detachment of eggs from leaves, (ii) biosynthesis of ovicidal compounds, and (iv) emission of leaf volatiles which attract parasitoids killing the eggs. Both the chemistry of egg-induced leaf volatiles and the parasitoid taxa responding to them show high diversity. Current studies of the molecular processes involved in egg-mediated plant reactions open numerous questions. So far, we know that the transcriptome of a plant exposed to egg depositions (without leaf wounding) differs from the one of an egg-free plant especially with respect to genes involved in the salicylic acid (SA) pathway. Interestingly, several plant species across various taxa intensify their defense against hatching larvae when having received insect eggs prior to larval feeding. In spite of slightly enhanced SA levels at the onset of larval feeding, previously egg-deposited plants reduce performance of herbivorous larvae more effectively than egg-free ones. This effect can be ascribed to enhanced larval uptake of phenylpropanoid plant compounds. In elm, we found that previously egg-deposited leaves respond earlier/faster to larval feeding damage than egg-free elm leaves. This result indicates that egg depositions preceding larval hatching shape the dynamics of plant responses to feeding damage.

Hilker, M. & Fatouros, N.E. (2016). Resisting the onset of herbivory: plants perceive and respond to insect eggs. *Curr. Opin. Plant Biol.* 32: 9-16.
Hilker, M. & Fatouros, N.E. (2015). Plant responses to insect egg deposition. *Annu. Rev. Entomol*. 60: 493-515..

Olivier Hamant

RDP - ENS Lyon, Fr

Mechanical signals in plant morphogenesis

In "On growth and forms" (1917), D'Arcy Thompson stresses the inevitable interactions between physics and biology. Thanks to ongoing developments in live imaging and modeling, this field of study has been rejuvenated: the relation between mechanics and shape changes can now be addressed more comprehensively, notably in plants in which morphogenesis is mainly determined by cell walls. In past work, we showed that shape- and growth-derived forces act as signals that orient plant microtubules. This response channels key biological features, such as cell shape, cell division plane orientation and final organ shape. Beyond microtubules, such forces also contribute to cell polarity and to the expression patterns of master regulators of meristem maintenance. The implications of this work are numerous and include a role of mechanical conflicts emerging from growth heterogeneity in the reproducibility of shapes. We are now addressing two major bottlenecks in the field: first, to formally challenge the role of mechanical signals in development, the relevant mechanotransduction pathways need to be identified; second, forces being invisible in essence, mechanical stress patterns in tissues need to be assessed experimentally, beyond computational model predictions.

Karin Schumacher

COS Heidelberg, DE

Vacuoles – pumping up the plant volume

The presence of a large central vacuole that fulfills multiple functions in storage, detoxification and cell growth is one of the hallmarks of a prototypical plant cell. Vacuolar transport is channeled by a battery of transport proteins that are all assumed to be energized by the combined activity of two proton-pumps, the vacuolar H+-pyrophosphatase (V-PPase) and the vacuolar H+-adenosinetriphosphatase (V-ATPase). In my presentation, I will discuss the physiological roles of the two proton-pumps, their trafficking routes to the tonoplast as well as the process of vacuole biogenesis.

Elizabeth S. Haswell

Washington U in St. Louis, MO, USA

Stretching the imagination: mechanosensitive channels in plants

A long-standing question is how biological systems sense and perceive mechanical signals such as osmotic pressure, gravity, and touch. One well-established molecular mechanism for force sensing is the activation of mechanosensitive (MS) ion channels. The Mechanosensitive channel of Small conductance (MscS) from *E. coli* functions as a hypo-osmotic safety valve, opening in response to increased membrane tension and preventing cellular rupture. Genes predicted to encode MscS homologs are found in genomes from all three kingdoms of life. We have been characterizing the structure, function, and regulation of ten MscS-Like (MSL) proteins in the model plant *Arabidopsis thaliana*. Based on their modest homology to MscS and high topological diversity, we have proposed that MSLs might (1) sense and respond to sources of membrane tension other than environmental hypo-osmotic shock; (2) be regulated by mechanisms in addition to membrane tension; and (3) signal in ways that are separable from ion flux. Evidence in support of all three of these hypotheses will be presented.

Elena M. Kramer

Harvard U, USA

Exploring the genetic basis of floral novelty in *Aquilegia*

The lower eudicot model system *Aquilegia* possesses several novel morphological features that have the potential to shed light on the evolution of novelty, particularly in the context of complex organ form. We have been studying two key features of the *Aquilegia* flower: the three-dimensional petal nectar spur and a fifth floral organ type, the staminodium. In the case of the petal spur, we have found that *Aquilegia* petal spurs initiate due to a localized region of cell division in which cell wall formation is radially organized around the presumptive nectary. This lays the ground pattern of the spur, which is then realized through rapid, anisotropic cell elongation that is the major determinant of spur length and curvature. Diversification of spur morphology has involved multiple factors, including heterochronic shifts that generate much longer, narrower cells; differences in cell numbers around the radial axis of the spur; and independent control of cell elongation on different surfaces of the spur, which generates curvature. We are now combining transcriptomics, candidate gene approaches, and QTL mapping to explore the genetic architecture of spur development and understand its evolution. In the case of the staminodium, we have previously discovered that this novel organ identity is determined via the combinatorial activity of paralogs of the genes that normally confer stamen identity, such that the stamens and staminodia now have distinct genetic codes via sub- and neofunctionalization. We are further exploring the developmental and morphological oddities of the staminodia, what their ecological function may be, and how their developmental program diverges from that of stamens.

Dolf Weijers

U Wageningen, NL

The *Arabidopsis* embryo as a model for understanding the genetic basis for multicellular development

Both growth and tissue patterning are processes that occur continuously during plant life. A key question is how these are coordinated in space and time to generate plant shape and function. We use the early *Arabidopsis* embryo as a simple and highly predictable model in which cell identity specification, growth and patterning are intricately coordinated. I will discuss our recent work aimed at understanding the cellular basis for the establishment of multicellular patterns in 3D. I will describe our efforts towards identifying the genetic and cellular basis for the establishment of cell polarity and oriented cell division.

Key recent literature:

ten Hove, C., Lu, K.J., and Weijers, D. (2015). Building a plant – cell fate specification in the early *Arabidopsis* embryo. *Development* 142, 420-430.

De Rybel, B., Adibi, M., Breda, A.S., Wendrich, J.W., Smit, M.E., Novak, O., Yamaguchi, N., Yoshida, S., Van Isterdael, G., Palovaara, J., Nijsse, B., Boekschoten, M.V., Hooiveld, G., Beeckman, T., Wagner, D., Ljung, K., Fleck, C., and Weijers, D. (2014). Integration of growth and patterning during vascular tissue formation in *Arabidopsis*. *Science* 345, 1255215.

Yoshida, S., Barbier de Reuille, P., Bassel, G., Lane, B., Prusinkiewicz, P., Smith, R.S., and Weijers, D. (2014). Genetic control of plant development by overriding a geometric division rule. *Dev. Cell* 29, 75-87.

Weijers, D., and Wagner, D. (2016). Transcriptional Responses to the auxin hormone. *Annu. Rev. Plant Biol*. doi: 10.1146/annurev-arplant-043015-112122.

Liao, C.-Y., Smet, W., Brunoud, G., Yoshida, S., Vernoux, T., and Weijers, D. (2015). Reporters for sensitive and quantitative measurement of auxin response. *Nature Methods* 12, 207-210.

Ralf Reski

U Freiburg, DE

Cuticle, sporophyte, stomata: three plant innovations that changed our planet

The conquest of land by plants occurred approx. 500 million years ago. Spread of the earliest land plants enhanced global photosynthesis capacity, which led to reduced CO_2- and elevated O_2-levels. The rapid decrease of CO_2-levels led to an ice age, while the increase in O_2-levels was crucial for the evolution of complex animals on land.
Based on our work with the model moss *Physcomitrella* patens I will present our recent findings on the molecular control of three major innovations that helped early plants to conquer the land masses and to spread relatively quickly. We discovered a P450 enzyme that is required for the formation of a phenol-enriched cuticle, which provides shelter against desiccation and facilitates the formation of complex plant organs, and was the progenitor of lignin. Further, we identified the homeobox transcription factor BELL1 as the first master regulator of embryogenesis (plants, animals, human) resulting in the formation of a diploid sporophyte; a finding that unravels the 166 year-old discovery of the alternation of generations common to all land plants. Finally, we describe two bHLH transcription factors as a basic genetic tool kit for the reprogramming of epidermis cells into stomata; microscopic valves of the plant surface that facilitate gas exchange with the environment.

Renault et al. (2017): A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nature Communications* 8, 14713.
Horst et al. (2016): A single homeobox gene triggers phase transition, embryogenesis and asexual reproduction. *Nature Plants* 2, 15209.
Chater et al. (2016): Origin and function of stomata in the moss *Physcomitrella* patens. *Nature Plants* 2, 16179.

Dominique Bergmann

Stanford U, USA

Making a (cellular) difference

Cell polarity can be manifested in the shape and activity of mature cells, or as a prerequisite for the asymmetric distribution of factors during formative cell divisions. Conserved "polarity complexes" coordinate divisions planes and unequal segregation of cellular components in animals, but the molecules and spindle-based mechanisms to achieve this are not conserved in plants. In plants, subcellular polarity is best described in terms of PIN and other integral plasma membrane proteins that transport hormones or metabolites in defined directions, or by the presence of plant-specific "polarity proteins" such as BASL that is localized during, and required for, asymmetric divisions of the stomatal lineage in the leaf epidermis. The stomatal lineage is an easily assessable and genetically tractable system for elucidating cell fate and polarity as asymmetric divisions and their consequences can be monitored over time in the developing tissue. I will present our latest work on BASL's partners and clients and how the *Arabidopsis* stomatal lineage might use a polarity complex to define fate and pattern.

Erik Nielsen

U Michigan, USA

Functional analysis of the roles of CSLD proteins during plant cell wall deposition in *Arabidopsis*

Plant cell expansion is governed by the deposition of new cell wall components, and cellulose microfibrils, the major load bearing components in plant cell walls, are deposited along one or more entire faces of a cell during diffuse growth. However, during tip growth, newly synthesized cell wall polysaccharides are deposited in a restricted region. Previously we have shown that the cellulose synthase-like D family protein, CSLD3, localizes to the tips of growing root hairs and provides essential cell wall synthase activity during root hair tip growth, and that fluorescently-tagged CSLD2/3/5 proteins localize to newly-forming cell plates in dividing cells. These results indicate that, in addition to their roles in cell wall deposition in tip-growing cells, CSLD proteins also provide essential cell wall synthase activity in cells undergoing cytokinesis. In both tip-growing cells and dividing cells, new cell wall deposition occurs in a highly polarized manner; either at the apex of the growing hair, or at the leading edge of the newly-forming cell plate. That CSLD proteins selectively localize to both these cellular regions may indicate that the cell wall polysaccharides synthesized by this class of cell wall synthases play important structural roles in these specialized zones of cell wall deposition. But what is the nature of the polysaccharide synthesized by CSLD proteins? We previously demonstrated that a chimeric CSLD3 protein containing the CESA6 catalytic domain rescues root hair defects observed in csld3 mutants, suggesting CSLD proteins may be glucan synthases. However, CSLDs have also been reported to have mannan synthase activities. In order to clarify the identity of the polysaccharides synthesized by CSLD proteins we have expressed CSLD and other plant glycan synthases in *S. cerevisiae*. CSLD3, CESA6, and CSLA9 were successfully expressed and purified using this heterologous expression system, and CSLD3 and CESA6 expressing microsomal membranes were determined to contain increased beta-(1—› 4)-glucan synthase activities. CSLD3, CESA6, and CSLA9 proteins were selectively extracted from yeast membranes using various detergents, and structural information regarding these protein complexes will be presented.

Poster abstracts

sorted by the order of submission

P1

Molecular mechanism underlying pollen tube reception

Andrea Zupunski, Hiroko Shimosato-Asano, Aurelien Boisson-Dernier, Heike Lindner, Ueli Grossniklaus

Institute of Plant and Microbial Biology, University of Zurich, Switzerland

First insights into the molecular basis of PT reception were provided by the discovery and characterization of the feronia (fer) mutant (Huck et al., 2003). FER encodes a receptor-like kinase (RLK) of the CrRLK1L subfamily (Escobar-Restrepo et al., 2007) and is widely expressed in plant tissues with its highest level in the synergids, but it is absent from pollen. Disruption of FER leads to PT overgrowth, where the PT continues to grow without bursting, resulting in a failure of fertilization. ANXUR1 (ANX1) and ANX2, the closest homologs of FER, have the opposite expression pattern to FER and are expressed only in pollen. PTs of anx1 anx2 double mutant burst prematurely and do not reach the ovules to effect fertilization (Boisson-Dernier et al., 2009). The goal of this project is to discover new molecular players present in the downstream signalling pathways. To address this question we are using both forward and reverse genetics approaches. We have already carried out forward genetic screen for mutants with a fer-like PT overgrowth phenotype and are currently analyzing previously uncharacterized mutants obtained in this screen. We want to make sure that the overgrowth phenotype is not due to the mutations in genes already described to be critical for this process (such as fer, nortia, lorelei, evan, turan). After excluding already identified mutations, we will identify new casual alleles using the SNP-ratio mapping approach (Lindner et al., 2012). We have also performed a supressor screen for anx1 anx2 double mutant phenotype which led to the identification of several novel factors downstream of the ANX1/2 kinases. We have identified homologs of these genes that are expressed in the female gametophyte based on microarray and RNA sequencing data. We are in the process of testing whether if disruption of these candidate genes will result in fer-like phenotype.

Keywords: feronia, double, fertilization, synergids

P2

Host Induced Gene Silencing in wheat powdery mildew

Luisa Schäfer

Institute of Plant and Microbial Biology, University of Zurich, Switzerland

Wheat powdery mildew is an important fungal pathogen of wheat. It can cause up to 40% yield loss. Virulence of this fungus depends on small secreted effector proteins which direct nutrient uptake from the host and suppress the plant immune response. Here, we show that Host Induced Gene Silencing (HIGS) of such an effector can cause quantitative disease resistance in wheat.

Keywords: HIGS, effectors, pathology

P3

Engineering plant disease resistance in the context of complex virus populations

Devang Mehta, Adrain Alder, Alessandra Stürchler, Matthias Hirsch-Hoffmann, Mariam Were, Andrea Patrignani, Hassan Were, Wilhelm Gruissem, Hervé Vanderschuren

Institute of Molecular Plant Biology, ETH Zurich, Switzerland
MMUST, Molecular Virology, Kenya
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Geminiviruses are circular ssDNA plant viruses that cause severe damage to major crops like tomato, maize, and cassava around the world. Here, we performed lab and field studies using RNA interference as a resistance mechanism to combat geminiviral disease in the staple food crop, Cassava. We also developed a new method for unbiased, single-read genome sequencing of circular viruses, called CIDER-Seq and applied this to study the impact of RNAi on virus populations in a field in Kenya. In an alternate approach, we created CRISPR-Cas9 expressing transgenic cassava plants to directly cleave geminiviral genomes in infected plants and used long-read sequencing to demonstrate the evolution of CRISPR-resistant viruses. We hence present an in-depth evaluation of RNAi and CRISPR technologies in engineering plant virus resistance.

Keywords: CRISPR, geminivirus, RNAi, PACBIO

P4

The single *Marchantia* CrRLK1L ortholog controls rhizoid formation and cell size during gametophyte development

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Unlike in animals, the life cycle of all land plants alternates between two multicellular generations, the haploid gametophyte and the diploid sporophyte. In the flowering plant *Arabidopsis thaliana*, many developmental aspects of fertilization, which marks the transition between the generations, are dependent on the joint functions of several members of the *Catharanthus roseus* RLK1-like (CrRLK1L) receptor-like kinase subfamily. The CrRLK1Ls, which also control diverse vegetative developmental processes, comprise 17 homologs in *A. thaliana*. Individual members can have very distinct, even opposing, developmental functions, making it difficult to assess a core function of the CrRLK1Ls. To reduce the genetic complexity, we explored the genome of *Marchantia polymorpha*, probably the most ancestral of land plants still extant today. Based on sequence homology, we identified a single CrRLK1L gene, named MpFERONIA (MpFER). Characterization of MpFER knockdown lines indicate that MpFER is involved in the vegetative development of M. polymorpha and controls rhizoid formation, overall growth and cell size. These phenotypes indicate a conserved and basal function of the CrRLK1L family in cell elongation. In addition, analysis of the MpFER expression pattern suggests further potential functions in the male and female sexual organ development, as well as sporophyte development. Thus, the core function of the CrRLK1L family originates from a single gene involved in cell elongation and has a conserved role in the vegetative development of the gametophyte. During land plant evolution, this ancestral gene was recruited, diversified, and specialized to fulfill new developmental roles in the formation of both gametophytic and sporophytic structures required in the angiosperm life cycle.

Keywords: CrRLK1Ls, *Marchantia*, evolution, ortholog

P5

Elucidate the role of post translational modifications on the dynamics of linker histones in the plant germline

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Chromatin is a complex of macromolecules and its organization is important for the regulation of gene expression, DNA replication and repair. Our group previously found that like in animals, plant chromatin undergoes large-scale remodeling during male and female germline differentiation. The hallmark of this process is a transient eviction of linker histones (H1's) from the germline precursor cells. However, the mechanism of H1 eviction and its role in germline differentiation remains unclear. The goal of our research project is to elucidate the role of post translational modifications (PTMs) on the mechanism of H1s eviction during plant germline differentiation. We adapted and optimized an inducible, molecular genetics system that allows to conditionally express H1 constructs with modified PTM sites. This allows to test for their importance in the regulation of H1 dynamics in the germline precursor cells in vivo. Preliminary results suggest that PTMs in the N-tail and globular domain of H1 are important for eviction and for chromatin compaction. We hypothesize that eviction of H1s in the germline precursor cell allows cellular reprogramming by providing competence to the chromatin to reprogram towards a reproductive lineage identity.

Keywords: *Arabidopsis*, histone

P6

RecQ helicases function in development, DNA repair and gene targeting in *Physcomitrella patens*

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A distinctive feature of the moss *Physcomitrella patens* compared to other plant model species is the high efficiency of gene targeting (GT). This competence for GT is based on homologous recombination (HR), a mechanism facilitating the recombination of genetic material during meiosis and the repair of DNA damages in somatic cells. RecQ helicases control recombination events like HR thereby contributing to the maintenance of genome stability and integrity. During evolution, the number of RecQ helicase-coding genes increased in accordance with the complexity of organisms along with a diversification of protein function. In the *Physcomitrella* genome six RecQ coding genes have been identified. In Arabidopsis two RecQ4 orthologs are present with antagonistic functions in DNA repair and HR. RecQ6 orthologs are present in all plants except the Brassicales, but their function was never studied until now. We generated a series of reporter and deletion lines of two of them in *P. patens*, PpRecQ4 and PpRecQ6, to study their role on plant morphology and development, DNA repair and GT. As *A. thaliana* and *P. patens* differ specifically in their RecQ4 and RecQ6 genes, we studied their gene functions in reciprocal cross-species approaches.

Keywords: evolution, recombination, gene-targeting, DNA-repair

P7

PTENs enzymes as novel regulators of xylem differentiation in *Arabidopsis thaliana* roots

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Phosphoinositides are very important membrane lipid signaling molecules that are known to regulate various cellular processes. Additionally, they confer identity to subcellular compartments and modulate protein trafficking within the endomembrane system. 3′ phosphorylated phosphoinositides are associated with the regulation of late endosome pathway as well as with vacuolar morphogenesis. In particular, recent data from our group has shown that an increase in the phosphoinositide-mediated vesicle trafficking towards the vacuole accelerates vacuolar biogenesis and, in turn, xylem differentiation. Therefore, to assess the importance of 3′ phosphorylated phosphoinositide species in vascular differentiation we aim at functionally characterising the enzymes involved in the 3′ phosphorylation of the inositol ring, the so-called PHOSPHATASE AND TENSIN HOMOLOG (PTENs). While overexpression of PTEN1 triggers a premature xylem differentiation, induction of PTEN3 expression negatively impacts this process by preventing vauolar biogenesis and secondary cell wall formation. As result, programmed cell death is interrupted in PTEN3-induced seedlings. PTEN3 localisation in the trans-Golgi network and its sensitivity to brefeldin A (BFA), an inhibitor of ARF-GEFs, which prevents vesicle trafficking at the level of TGN, suggest a potential role of PTEN3 in subcellular trafficking. Our results reinforce the idea that proper vacuolar morphogenesis is critical for proper xylem differentiation. Further analysis of PTENs expression patterns, subcellular localisation as well as their biochemical activity will improve our understanding about 3′ phosphate species in vacuolar morphogenesis. Finally, the establishment of a PTEN3-dependent molecular functional network will improve our understanding about the spatio-temporal regulation of the vesicle trafficking to guarantee an optimal xylem differentiation.

Keywords: PTEN, phosphoinositides, xylem, vacuole, secondary cell wall, programmed cell death

P8

Are sRNAs involved in the drastic shift of allele-specific expression in hybrid tomato seeds?

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A transcriptomic analysis of endosperms from intra- and interspecific crosses was performed. We found evidence of genomic imprinting in the endosperm of Solanum, identified a strong misregulation of gene expression in hybrid endosperms, and a genome-wide shift toward higher maternal expression proportions in hybrid endosperms that we posit is due to a failure of RNA-based silencing mechanism. A by-product of this higher expression proportion from maternally derived alleles is a close-to-total loss of paternally expressed genes in the endosperm of hybrid seeds. Based on these data, we hypothesize that siRNA-based silencing mechanisms, specifically the RNA-directed DNA methylation (RdDM) pathway, may be responsible for the upregulation of maternal alleles, among other transcriptional disturbances. The strongest indication of methylation involvement is the upregulation of MADS-box genes in hybrid endosperms. If the regulation of MADS-box genes in wild tomato species follows the same pattern as that in *Arabidopsis*, we expect to see siRNAs to be in lower abundance in hybrid endosperms and this in turn lead to lower methylation levels. Here we partly test this hypothesis and find that differentially expressed siRNAs were downregulated in hybrid seeds, but the question of the methylation levels remained unsolved. We found clusters of siRNAs in the vicinity of transposases and dicer coding genes, pointing to an involvement of the RdDM machinery in siRNA expression. These interesting results call for further investigations and in particular to elucidate how the methylation marks are set upon siRNA expression. Our research outlook is to complement the small RNA results by correlating methylation and siRNA expression in order to find causality to hybrid seed failure. Our analyses could also unravel more basic epigenetic mechanisms since these have never been studied in Solanacea.

Keywords: endosperm, siRNA, tomato

P9

The endodermal passage cell – just another brick in the wall?

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To maintain a sufficient transport of water and nutrients from the soil, it is crucial for plants to have an efficient barrier system that facilitates uptake and prevents backflow. In old roots, cork-like suberin lamellae are deposited in the cell walls of the endodermis, which is believed to serve as a barrier for transcellular transport. While this deposition occurs in the majority of old endodermal cells, those adjacent to xylem poles do not always undergo suberization. These cells are historically characterized as "passage cells" based on the assumption that they retain capacity for transport in an otherwise sealed area. While occurring in a range of plant species the term passage cell is ill fitting, as our knowledge is purely descriptive. By developing markers that highlight passage cells we discovered that these cells respond to local auxin/cytokinin ratio changes in the nanomolar range, as well as to non-cell autonomous inhibition of cytokinin signaling in the meristem. We found that passage cells are producers of cytokinin while at the same time refractory to cytokinin signaling, which suggest that they share developmental features with xylem. By investigating the expression of nutrient transporters, we emphasize that not only are passage cells a distinct cell type, they likely constitute a nucleation point for trans-cellular channeling of substrates from the rhizosphere to the vasculature. Based on our findings, we argue that not only does the xylem pole endodermis undergo individual programming; the well-established bilateral symmetry of the root stele should be expanded to include cortical layers.

Keywords: development, differentiation, nutrients, hormones, physiology

P10

Analysis of phosphatidylinositol4,5 bis-phosphate (PIP2) involvement in shaping different aspects of vascular formation in *Arabidopsis thaliana*

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The vascular system of plants is formed by two tissue types: xylem and phloem, responsible for water and photoassimilate transport respectively. This vascular network in cotyledons and leaves is specified from a common precursor, the probambium although the mechanisms responsible for vascular formation in these organs are still largely unknown. Recently, two enzymes involved in the phosphoinositide biosynthetic pathway, COTYELDON VASCULAR2 (CVP2) and CVP2 LIKE1(CVL1) have been described to regulate vein patterning and vascular tissue differentiation. These enzymes catalyze the conversion of phosphatydilinositol (4,5)bisphosphate(PIP2) into phosphatydilinositol4-phosphate(PIP).The cvp2 cvl1 double mutant exhibits a short root, protophloem defects and vastly discontinuous cotyledon vein patterning. Utilizing the cotyledon as a model for studying vascular development and vein patterning in combination with genetic, cell biology and transcriptomic approaches I will identify PIP2 downstream targets that are involved in determining the final architecture of vasculature.

P11

Multiple hybrid *de novo* genome assembly of an allotetraploid crop, finger millet

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It is a challenge to assemble a highly heterozygous or polyploid genome. The heterozygosity or polyploidy of genome causes a lot of homologous sequenced reads and it makes complicated to concatenate short reads in most cases of graph based *de novo* assembly method. Typically domesticated crops such as rice, wheat, and finger millet are mostly polyploid plants, and it is said that about 30-80% of living plants are polyploid. We constructed a hybrid *de novo* assembly pipeline for a polyploid genome combining different technology data: 1) Illumina short reads, 2) PacBio long reads, and 3) Bionano optical genome mapping data, and assembled the genome of an allotetraploid crop, finger millet (*Eleusine coracana* (L.) Gaertn). The total number of scaffolds was 1,897 with an N50 length >2.6 Mb and detection of 96% of the universal single-copy orthologs. The majority of the homeologs were assembled separately. Additionally, with its parental genome, we succeeded in the homeolog phasing.

Keywords: hybrid *de novo* genome assembly, polyploid plant genome, homeolog separation

P12

Periderm development: a tale of growth and programmed cell death

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The periderm is a frontier tissue and its main function, similarly to the epidermis during primary development, is to protect the plant against biotic and abiotic stress. Similarly to vascular system, the periderm comprises three tissues: the phellogen/cork cambium that produces inward the phelloderm and outward the suberized phellem/cork. Despite the economical and agronomical relevance, the molecular network underlying periderm establishment is largely unknown. To shed light on the mechanisms of periderm formation we established a framework to study periderm growth in *Arabidopsis* and developed a suite of tools. We identified 6 distinct stages of periderm growth, taking in consideration periderm ontogenesis and the fate of the surrounding tissues. In fact, we showed that periderm growth is tightly connected to the development of the outside tissues and in particular to endodermal programmed cell death. We also investigated how lateral root formation and periderm growth are connected as both originate from the pericycle. Mutants incapable of forming lateral root or blocked in lateral root emergence formed a normal periderm, suggesting that the two processes are independent. In addition, we will report weather the phellogen shares some key regulators within the vascular cambium network and how these tissues influence each other growth.

P13

The role of cell identity in the response to cell wall perturbation in *Arabidopsis thaliana* root

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Root growth depends on tight co-ordination between different tissues, which involves cell elongation, proliferation and differentiation. These processes require constant cell wall biosynthesis and remodeling throughout the plant life cycle. Therefore, close surveillance of the cell wall status is essential to ensure the regular growth of the root. By using *Arabidopsis thaliana* seedling primary root as research model, previous studies in our lab allowed the identification of a novel signaling pathway that ensures cell wall homeostasis by monitoring the state of pectin in the cell wall and regulate root growth through receptor like protein 44 (RLP44)-mediated activation of the brassinosteroid (BR) signaling pathway. We then triggered the perturbation of cell wall with mis-expression of pectin methylesterase inhibitor 5 (PMEI5) driven by a set of tissue/cell type-specific promoters in an inducible system, which allowed the investigation of the contribution of tissue/cell identity to the cell wall surveillance and the tissue/cell type-level influence of cell wall status on root development. By following responses on cellular, tissue and organ level, we observed that triggering cell wall perturbation in trichoblast cells, xylem pole pericycle cells and vasculature had the same organ morphogenesis outcome such as primary root waving. More over, perturbing cell wall in the cortex caused abnormal cell division and tissue organization *per se* and in neighboring tissues, which suggested the existence of both cell- and non cell-autonomous response. Interestingly, cell wall perturbation triggered in the cortex also resulted in a disrupted stem cell niche organization and the lost of quiescent center identity. These phenotypes did not seem to only result from the same RLP44- and BRI1-dependant regulatory mechanism, which raised the possibility that other unknown cell wall-mediated signaling pathways might also be in play.

Keywords: cell, wall, cell identity, root, development

P14

Research into the function of RubisCO in green seeds

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Seeds from a great number of plants, including many grown for agricultural purposes are green during their development. The aim of the research is to test genetically a pathway that was proposed to improve efficiency of lipid synthesis during the development of green seeds.

Keywords: Calvin-Benson cycle, lipid, seed, *Arabidopsis*

P15

Genome-wide perturbations of gene expression and parental dosage in wild tomato hybrid endosperms

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Endosperm failure is a suspected driver of seed abortion common in hybrid crosses. Differences in parental dosage and genomic imprinting (i.e., the parent-of-origin–dependent expression) have been proposed as potential factors for endosperm failure. We exploited variable severities of hybrid seed failure among wild tomatoes (*Solanum section Lycopersicon*) to explore associations between global expression levels, parent-specific gene expression, and imprinting. Based on transcriptome sequencing of developing endosperm from crosses within and among three wild tomato lineages, we uncovered strong associations between gene expression perturbation and the degree of hybrid seed failure. Importantly, a general increase in maternal expression proportions characterized endosperms from strongly abortive crosses involving *S. peruvianum* and either *S. chilense* or *S. arcanum var marañón* as the other parent. Altered parental expression proportions in hybrid endosperms of these two lineage pairs led to widespread loss of imprinting status, particularly for paternally expressed genes. At genome-wide scales, the reciprocal crosses of these abortive pairs were separated by the largest total expression differences in the entire data set, corresponding to substantial phenotypic asymmetries in terms of endosperm development and mature seed size. We accrued evidence that impaired auxin control may mediate the curtailed or prolonged endosperm proliferation phase that drives hybrid asymmetries in endosperm and mature seed size. Our data suggest that differences in parental dosage may underlie the perturbation of dosage-sensitive pathways. In particular, misregulation of AGAMOUS-LIKE transcription factors may directly cause auxin imbalance. Consistent with predictions of the kin conflict theory, we hypothesize that tomato lineages evolved different 'genetic strengths' as an evolutionary response to dissimilar levels of parental conflict after their divergence.

Keywords: endosperm, hybrid, tomato, imprinting, abortion

P16

DEX-inducible reporter lines for spatio-temporal cell type-specific expression in *A. thaliana*

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The introduction of transgenes in the early 80s opened the door to scientists for genetic engineering. The usage of this had substantially contributed to our understanding on the mechanisms that occur during plant development. It has been extensively used general-purpose constitutively promoters to drive the expression of transgenes. But this often can lead to an out of context functions or even for certain genes compromise plant viability. Therefore, it has been of increasingly importance to study gene function in its natural context by the use of its own promoters. Additionally, gene function can be different depending on its spatial or temporal distribution, for example, interacting partners present only in a specific cell-type or those that require an external cue to be expressed. Therefore, the use of a conditional and cell-type specific control of transgene expression can provide a detailed temporal-spatial resolution to study gene function. Here we present a toolbox of 31 transgenic lines to study gene function in a spatio-temporal resolution that comprehensibly cover most of *Arabidopsis* tissues. These where generated with a cassette that comprises of a cell-type specific promoter that drives the expression of the LhGR (DRIVER lines) and four copies of the pOp (p6xOP) promoter that drive the expression of the mTurquoise2 fluorophore to monitor the spatio-temporal specificity of the induction. With these lines as tools, by introducing a transgene that carries the p6xOP with any gene (EFFECTOR), it can be investigated the gene function or its contribution in a specific tissue to overall plant architecture.

Keywords: *Arabidopsis*, development, inducible, gene-function

P17

Interplay between auxin and phosphoinositides in regulation xylem differentiation in *Arabidopsis thaliana* roots

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Plant vasculature is formed by xylem tissues, which delivery water and nutrients from the root to the photosynthetic organs, and phloem elements responsible for the transport of synthesized sugars throughout the whole plant body. Xylem differentiation is a complex process encompassing several steps, from secondary cell wall formation (SCW) to eventual programmed cell death (PCD), and cell wall lignification. Recently, our group showed the role of phosphatydilinositol (4,5) bis-phosphate [PtdIns(4,5)P2], a lipid compound virtually present in all cell membranes in regulating xylem differentiation. In particular we observed that a xylem specific increase of PtdIns(4,5)P2 enhances vesicle trafficking towards the vacuole leading to an early protoxylem appearance, as observed by a premature vacuolar rupture, SCW formation and PCD. Interestingly, this process appears to be associated with auxin activity, since an inhibition of auxin perception delays protoxylem differentiation. While auxin is known to regulate vacuolar morphology in epidermal cells, its role in the diverse subcellular processes occurring during xylem differentiation remains still obscure. We are now exploring the hypothesis of an interplay between auxin and PtdIns(4,5)P2, aiming at unravelling a new phosphoinositide-dependent mechanism in xylem differentiation.

Keywords: auxin, PtdIns(4;5)P2, xylem, differentiation, trafficking

P18

Spatial control of cell growth and tissue differentiation with 3D printing

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Root stem cell maintenance and identity is controlled by signals from the local micro-environment. Despite much advancement in our understanding of how specific gene networks are working to control individual stem cell types, an understanding of cell-to-cell communication and spatial orientation essential for maintaining stem cells and regulating cellular differentiation is lacking. Plant cells can be easily isolated and manipulated because they do not move. Moreover, root stem cells divide in a stereotypical manner and their tissues are organized into cell layers where entire cell lineages are spatially restricted. Despite this great advantage, 3D-printing technologies have been limited to animal tissues. We have utilized cutting edge 3D-bioprinting technology to precisely distribute plant cells, we have developed protocols to quantitatively measure cell viability and cell wall formation in control and in 3D-bioprinted cells. We have developed a mathematical model that predicts the desired traits based on the spatio-temporal placement of cells. We propose that by using 3D-printing we can test critical interactions of cells within the niche and redesign novel plant systems.

Keywords: 3D-Bioprinting, root, stem cells

P19

A single dominant mutation conferred self-compatibility in allotetraploid *Arabidopsis kamchatica*

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The genetic basis of the recurrent evolutionary transition from outcrossing (self-incompatible) to selfing (self-compatible) has been a major focus in evolutionary biology. This transition might occur if plants experienced unsuitable conditions, e.g., during migration when pollinators or mates are scarce, because of the "reproduction assurance" that could be gained through selfing. On the other hand, polyploids, which are commonly found in plants, are suggested to self more frequently than their diploid relatives. However, the underlying mechanism is still largely unknown. Here, we studied the loss of self-incompatibility in *Arabidopsis kamchatica*, which is a selfing allotetraploid species, originated through allopolyploidization between two predominantly outcrossing diploid species, *Arabidopsis halleri* and *Arabidopsis lyrata*. We applied high-throughput sequencing to isolate the male component, S-locus cysteine-rich protein (SCR) genes and detected the presence of gene-disruptive mutations in SCR genes of *A. kamchatica*. Transgenic method of *A. kamchatica* was developed to functionally validate the roles of SCR genes in *A. kamchatica*. We showed that the loss-of-function mutation in the dominant male component contributed to the evolution of selfing in allotetraploid *Arabidopsis kamchatica*.

Keywords: sRNA, dominance, allotetraploid, self-incompatibility

P20

Biofortification of staple cereals – addressing micronutrient deficiencies affecting human health

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Micronutrient deficiencies, especially those of iron, zinc and vitamin A, are prevalent worldwide and are among the most critical challenges to human health today. Biofortification, an attempt to increase the micronutrient concentration within the edible parts of the crops, holds a great potential for addressing micronutrient deficiencies. By introducing multiple exogenous genes that contribute to iron uptake, transport and storage into one locus, we have developed rice and wheat lines that have significantly increased concentration of iron and zinc. Furthermore, by introducing pro-vitamin A synthesis genes together with the genes for iron and zinc biofortification into one locus, we have created rice lines with high provitamin A concentration in the grains besides high iron and zinc concentrations. We continue to optimize our strategies to transfer improved grain micronutrient traits to breeding preferred cultivars and we are also developing novel biofortification approaches involving state-of-the-art gene-editing technologies.

Keywords: iron, zinc, rice, wheat, biotechnology

P21

A specific quorum sensing dependent molecule inhibits *Arabidopsis* germination through DELLA and ABA signaling

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Germination is a tightly controlled developmental process transforming the highly protected embryo within the seed into a fragile seedling. Plants have evolved control mechanisms to block germination under unfavorable conditions to avoid potentially fatal conditions for the seedling. Control of seed germination was mainly studied in the context of abiotic stresses such as poor light quality (canopy light), high temperature or salinity. Abiotic parameters ultimately control the activities of the GA and ABA signaling pathways that promote and repress seed germination, respectively. Unfavorable abiotic conditions lead to low GA synthesis. GA promotes the destruction of the so-called DELLA factors that promote endogenous ABA accumulation, which blocks germination. However, in nature seeds are continuously exposed to organisms such as bacteria, fungi or nematodes, which can be potential pathogens. We observed that the plant pathogen *Pseudomonas aeruginosa* secretes an activity that represses *Arabidopsis* seed germination. We show that this biotic activity promotes DELLA accumulation and ABA signaling irrespective of GA levels. Metabolomic and bioguided biochemical fractionation approaches led us to identify an oxyvinylglycine as the main germination repressive activity (GRA) released by *Pseudomonas* repressing seed germination. Quorum sensing (QS) is a bacterial system regulating the expression of numerous genes to mount coordinated behavioral responses in the bacterial population. Interestingly, we found that the GRA is controlled by a quorum sensing system that is specific to *Pseudomonas aeruginosa*. Altogether, our data suggest that in the course of evolution, plants have evolved mechanisms to responds to biotic signals betraying the presence of potentially harmful pathogens.

Keywords: DELLA, ABA, seed, germination, *Pseudomonas*

P22

LRX: an extracellular protein required for cell wall development

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Each plant cell is surrounded by a rigid yet flexible cell wall that protects and provides stability to the cell. During development, the cell wall is constantly modified and turned over to expand and adjust to the requirements or respond to biotic and abiotic stresses. While the composition of the cell wall is quite well understood, the regulatory mechanisms controlling cell wall composition and modification are not well elucidated. We are investigating the function of LRX proteins, which are extracellular components involved in cell wall development. These LRR-extensins serve a regulatory or signaling function and their absence impacts cell wall structures and, consequently, cell and plant growth. The extensin domain anchors the protein in the cell wall while the LRR domain appears to be involved in protein-protein interaction. I aim to elucidate the chemical nature of the interaction of the extensin domain with the cell wall matrix. Further, we are trying to identify the interaction partner of the LRR domain, which should ultimately provide an insight into the process(es) LRX proteins are involved in.

Keywords: LRX, cell wall, extensin, glycosylation

P23

Protein networking: novel actors in starch metabolism

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Transitory starch is a plant storage compound produced as insoluble, semi-crystalline granules in chloroplasts during the day and degraded to provide energy and carbohydrate to sustain metabolism at night. Starch consists of two glucose polymers - amylopectin and amylose - that differ in structural complexity. Amylose is an essentially linear polymer, synthesized by a single granule bound starch synthase (GBSS). Amylopectin is a branched polymer the specific architecture of which is determined by the activities of several soluble starch synthases (SSs), branching enzymes and debranching enzymes. While these individual steps are relatively well understood, recent investigations have shown that starch metabolism entails more than just the sum of these enzymatic activities. We have identified several previously uncharacterized proteins that are essential for normal starch metabolism. Our data suggest that these proteins interact in networks and that dynamic protein-protein interplay is required to control the complex processes of starch granule formation. We are implementing a combination of approaches, including bioinformatics, in vitro and in vivo assays, and the analysis of transgenic *Arabidopsis thaliana* lines to characterize these proteins - and their relations to each other - in detail. This will help us to shed light on aspects of starch metabolism that have until now remained shrouded.

Keywords: starch, carbohydrate, metabolism, *Arabidopsis*

P24

Improving disease resistance of pea – clues from plant-microbe interactions

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Pea (*Pisum sativum* L.) is a valuable protein source for food and feed. Pea is able to significantly improve soil fertility and, hence, represents an ecologically important crop in low-input farming systems. Despite their importance, pea cultivation remains below expectations due to low and unstable yields caused by a complex of soil-borne pathogens. The goal of this project is to improve our understanding of resistance mechanisms of pea against soil-borne diseases. To achieve this goal, more than 300 pea lines were evaluated for resistance in pot-experiments and a subset of susceptible and resistant pea genotypes were identified. In a next step, key pathogens and beneficials in the pea rhizosphere and the role of root exudates in determining the occurrence of these microbes will be investigated. The study will shed light on the complex interactions between pea genotypes and soil microbes, and promote resistance breeding programmes for legumes.

Keywords: *Pisum sativum*, sustainable agriculture, soil-fatigue, plant-microbe interaction, root exudates, genetic resources, breeding

P25

Untying the loop: the role of Loop>J in ARF-GEF function

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Fundamental processes like cell-cell communication, signaling and the specific distribution of proteins within a cell rely on an efficient membrane transport machinery. Therefore, the specific regulation of vesicle trafficking processes through ARF-GTPases, ARF-GEFs (Guanine-nucleotide-Exchange Factors) and coat proteins is essential for eukaryotic organisms. ARF-GEFs mediate ARF-GTPase activation, leading to coat recruitment and consequently to vesicle budding. Large ARF-GEFs comprise five non-catalytic protein domains in addition to the highly conserved catalytic SEC7 domain (Anders et al. 2008). A region of the SEC7 domain named "loop>J" has been shown to play a role in the catalytic activity of ARF-GEFs in animals (Lowery et al., 2011). Because in plants, membrane association of ARF-GEFs such as GNOM requires the heterotypic interaction of the DCB domain with HUS and SEC7 domains, we investigated whether the loop>J (Jl) exhibits additional features in plant ARF-GEFs (Anders at al., 2008). The GNOM-Jl3xA mutant protein was studied in regard to subcellular localization, GNOM dimerization, membrane association, functional requirement and ARF1 interaction in *Arabidopsis thaliana*. So far, no defects in membrane association or dimerization were observed. Nevertheless, the interaction between GNOM-Jl3xA and ARF1 seems to be impaired, resulting in only partially rescued gnom knockout seedlings. Our results suggest that ARF-GEF binding to the membrane is independent of the interaction between ARF-GEF and ARF-GTPase but this needs to be confirmed in future studies.

Keywords: vesicle, trafficking, ARF-GEF, GNOM

P26

Genomic tools to assess within- and between-species diversity in grassland plants

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Grasslands are widely distributed ecosystems, which in temperate latitudes provide the basis for sustainable ruminant nutrition. Grasslands also contribute to the maintenance of overall biodiversity, fulfil many ecological services, and harbour valuable genetic resources for the improvement of forages. Grassland plant biodiversity is therefore a valuable resource that needs to be preserved. Our aim is to develop high-throughput methods to assess species richness and to measure within-species genetic diversity in grassland plants. We focus on 16 key grass and legume species (SOI-16) commonly found in Swiss grasslands; however, the methods should also be applicable in other temperate grasslands. The methods that we are assessing are plant DNA barcoding (for species-level taxonomic assignments, or SLTA for short) and multi-locus amplicon sequencing (for genetic diversity measurements). Plant DNA barcodes are mostly regions of the chloroplast genome that are useful for SLTAs. The Barcoding of Life Database (BOLD) is an effort to compile worldwide DNA barcoding data of living organisms and mantains a barcode reference database. We amplified and Sanger-sequenced four plant DNA barcodes (rbcLa, matK, trnH-psbA and psbK-psbI) from different varieties of the SOI-16. SLTAs were perfomed using a reference database with the sequences of the Poaceae and Fabaceae entries in BOLD; we used a custom classification method based on species-by-species alignments and weight-matrix scoring. We found that barcode trnH-psbA and its concatenation with rbcL accurately assign most of the analysed samples. However, some grasses consistently generated ambiguous SLTAs. Finally, in order to measure within-species genetic diversity, we identified 611 single-copy, orthologous genes and ultra-conserved elements. These target-loci will be enriched through sequence capture and sequenced using the Illumina MiSeq; the most informative loci will then be selected to develop a PCR-based method.

Keywords: molecular ecology, barcoding, forages, sequence capture, NGS

P27

Molecular correlates of temperature-induced sex reversal and genetic improvements to adapt to changing climate

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Carica papaya L. is a trioecious crop species that is cultivated in tropical and subtropical regions around the world. Only female and hermaphroditic individuals produce fruit, and growers in the Americas prefer to grow hermaphrodite plants due to the advantages of self-pollination and higher crop uniformity. However, certain environmental conditions, such as elevated temperatures, cause sex reversal in hermaphroditic plants, leading to the development of male flowers that do not produce fruits. Therefore, sex reversal results in economic damage for papaya growers. The molecular mechanism behind sex reversal remains unknown, and the aims of this project are the investigation of the molecular factors that cause sex reversal and the development of molecular markers that allow breeding for varieties with a more stable sex expression. We used microscopy techniques to determine the critical period for sex determination during flower development. We plan to isolate nuclei from female, hermaphrodite, and sex reversed floral buds at the critical stage for single nuclei RNA-seq to identify potential candidate genes related to sex reversal. In a second part of the project, we will screen different cultivars of papaya to identify varieties that are less susceptible to sex reversal and try to find associated markers which could be used in future breeding efforts.

Keywords: *Carica*, papaya, sex-reversal, RNA-seq, sex-expression

P28

Mechanistic basis for the activation of plant membrane receptor kinases by SERK-family co-receptors

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Many LRR-RKs require SERK-family co-receptor kinases for high-affinity ligand binding and receptor activation. How one co-receptor can contribute to the specific binding of distinct ligands and activation of different LRR-RKs is poorly understood. Here we quantitatively analyze the contribution of SERK3 to ligand binding and activation of the brassinosteroid receptor BRI1 and the peptide hormone receptor HAESA. We show that while the isolated receptors sense their respective ligands with drastically different binding affinities, the SERK3 ectodomain binds the ligand-associated receptors with very similar binding kinetics. We identify residues in the SERK3 N-terminal capping domain, which allow for selective steroid and peptide hormone recognition. In contrast, residues in the SERK3 LRR core form a second, constitutive receptor – co-receptor interface. A functional BRI1 – HAESA chimaera suggests that the receptor activation mechanism is conserved among different LRR-RKs, and that their signaling specificity is encoded in the kinase domain of the receptor. Our work defines the relative contributions of receptor, ligand and co-receptor to the formation and activation of membrane signaling complexes regulating plant growth and development.

Keywords: brassinosteroid signaling, floral abscission, membrane receptor kinase, leucine-rich repeat domain, receptor activation, brassinosteroid signaling, floral abscission, membrane receptor kinase, leucine-rich repeat domain, receptor activation

P29

Elucidation of the TOR (target of rapamycin) network by new TOR kinase alleles

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The target of rapamycin (TOR) is a highly conserved Ser/Thr kinase central to the TOR network. Manipulation of the TOR signaling network in *Arabidopsis* has highlighted its central role in regulating translation and metabolism, as well as cell proliferation and cell wall integrity. The lethality of the AtTOR gene disruption called for alternative strategies to characterize the functions of this gene. The application of chemical inhibitors with specificity to the TOR kinase, as well as TOR silencing in transgenic *Arabidopsis* based on RNA interference have proven to uncover diverse biological plant TOR functions. However, prolonged chemical treatment and variable efficiency of TOR silencing might induce a range of phenotypes, which could hinder interpretations of TOR signaling functions. In this project two newly identified tor alleles, harboring a missense mutation in the TOR kinase domain, are investigated. Phenotypic analysis of the tor alleles reveals a consistent alteration of root growth. The tor alleles display different sensitivities to TOR kinase inhibitors compared to the wild type. Therefore, further analyses of the two tor alleles will provide insight in the role of the TOR pathway in controlling plant growth. This will potentially help to establish means to modify plant development including biomass production and plant yield.

P30

Integration of two signalling pathways controls vascular cell fate in the *Arabidopsis* root

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Plants are multicellular organisms consisting of millions of cells with discrete functions. Like in animals, cell fate determination mainly depends on integration of intrinsic and extrinsic cues such as clonal cell identity and positional information acquired through non-cell autonomous signals. Here, we study the differentiation of xylem cells, which is a terminal differentiation that involves irreversible cellular events like programmed cell death and secondary cell wall formation. RECEPTOR-LIKE PROTEIN 44 (RLP44) is mainly expressed in the vascular tissue, predominantly in the procambial cells, and so far it is known to be involved in cell wall mediated activation of the brassinosteroid signalling pathway. In this work, we show that balancing between two signalling pathways is crucial for the root vascular cell fate determination. A shift in this balance results in a reorganized vascular composition. The critical role is taken by RLP44 which can directly interact with two different receptor kinases, and consequently integrates two independent downstream signalling pathways. With a newly identified bri1 (BRASSINOSTEROID INSENSITIVE 1) mutant, named bri1cnu4, we can provide data for the possible mechanistic regulation. This mutant is not severely affected in the BR-sensitivity and allows us to study the changes in the direct interaction between RLP44 and BRI1. We provide evidence that the interaction between RLP44 and BRI1cnu4 is enhanced and, as a consequence, RLP44 is tethered away from a second RLK interaction partner. Our data support the model that this BR-independent pathway is important for the determination of the procambial identity. Therefore, the dynamic balancing of the two pathways may be a key regulatory step of xylem development.

Keywords: cell fate, xylem, procambium, signalling, cell wall development, differentiation, root

P31

Shared polymorphism, demography and purifying selection in diploid and allopolyploid *Arabidopsis* species

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Comparisons between diploid parents and their derived subgenomes can be used to test the effects of genome duplication by allopolyploidy. *Arabidopsis kamchatica* is a self-compatible (SC), natural allotetraploid derived from the self-incompatible (SI) *A. halleri* subsp. *gemmifera* (found in E. Asia) and with *A. lyrata* subsp. *petraea* (from Far East Russia). A transition from SI to SC accompanied by whole genome duplication is expected to result in a large reduction in genetic diversity compared with the diploid relatives. We used demographic models to better understand the complex hybridization history of *A. kamchatica*, and the coalescence of the subgenomes to the parental species. Genome wide diversity of the two subgenomes is about 5 times lower than either diploid parent. Shared polymorphism with the diploids is about 40% of SNPs shared between the subgenomes and their corresponding parents. Weaker purifying selection and thereby an increase in relaxed constraint, is a predicted result of whole genome duplication due to masking of deleterious mutations by functionally redundant gene copies. This should be evident by comparing the proportions of neutral vs. deleterious mutations in the polyploid and diploid genomes. The estimated DFE of the diploids show lower proportions of neutral mutations, and greater proportions of deleterious mutations than either of the corresponding subgenomes. While the differences are significant, the differences are not remarkable. We estimated the proportion of adaptive substitutions in both subgenomes the two diploid species. For the diploid species, was significantly greater than zero with *A. halleri* and *A. lyrata* showing the highest values (0.25-0.27). Both subgenomes of *A. kamchatica* also had significantly positive and indicated 6-12% of non-synonymous substitutions are adaptive. The positive values of *A. kamchatica* are about half of the diploid species which most likely reflects genome duplication and a reduction in Ne.

Keywords: demography, selection, diversity, allopolyploid

P32

How comparable is a cisgenic apple line to its original germplasm and to natural mutants of the same cultivar?

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Most apple cultivars grown for commercial purposes are highly susceptible to several diseases and require high chemical inputs to produce fruits of commercial quality. Disease resistant cultivars are currently developed by conventional breeding but still fail to outcompete well established susceptible cultivars. An alternative approach based on genetic engineering was used to improve the popular variety 'Gala Galaxy' by a single integration of the endogenous fire-blight resistance gene FB_MR5 on chromosome 16 which resulted in the cisgenic fire-blight resistant apple line C44.4.146. The cisgenic approach is expected to add new traits to a cultivar without changing its beneficial qualities. To verify this, a field trial was set up to compare C44.4.146 to 'Gala Galaxy' which is a bud sport of the variety 'Gala'. In the same field trial also 'Gala' and two other bud sports of 'Gala' have been included. Sports are generally considered as safe as the cultivar they derive from, even if the underlying changes have not yet been understood. This setup allows to compare the effect of the cisgenic approach to the effect of naturally occurring mutations from an agronomical but also from a multi-omics perspective. For this latter, whole genome sequences will be compared to call structural and sequence variants among sports and assess if the cisgenic approach induced other changes than the desired cisgenic insertion. Further transcriptome, proteome and metabolome data will be generated from the same leaf samples and compared. The broadness of biological molecules investigated gives a unique opportunity to investigate the molecular changes induced by the cisgenic modification and assess their relevance in the context of natural variability among 'Gala' sports. Moreover, the data offer an unprecedented opportunity to integrate the results of the different -omics approaches.

Keywords: genomics, transcriptomics, proteomics, metabolomics

P33

Using mechanics to uncouple the processes of development

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The spatial pattern of mechanical properties across tissues controls morphogenesis in plants. To investigate these spatial patterns in the growing, developing tissues of the model plant *Arabidopsis* I developed an automated confocal micro-extensometer (ACME). ACME enables forces to be applied to tissues, while they are imaged with a confocal microscope. These images can be analysed using the image analysis software MorphoGraphX to extract 3D cellular strain measurements. This has enabled us to reveal spatial gradients in mechanical properties that correlate with the pattern of gibberellic acid-induced growth. In addition to measuring mechanical properties, we used ACME to investigate responses to mechanical stress, which has recently re-emerged as an important determinant of plant development. We were able to show that microtubules reorient in response to forces within the physiological range. However, the response was dependent upon the direction in which the force was applied.

Keywords: development, mechanics

P34

A role for GDSL lipases/esterases in the development of functional stomata?

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Gene duplication events lead to gene families with similar biochemical functions in all living organisms. One such gene family is the GDSL-like lipase/esterase family, which consists of 100 members in *Arabidopsis thaliana*. This group contains the specific and highly conserved GDSL motif. GDSL-like lipases are described as lipolytic enzymes, however, our knowledge of their exact function is still limited. We are investigating the functional role of two of the members, CGM3 and CGM4 (Contains GDSL Motif). We found that CGM3 and CGM4 are localized in the apoplast and promoter analysis showed that CGM4 is specifically expressed in early meristemoid cells of the stomatal lineage. In spite of this specific expression pattern, the single mutant lines of cgm3 or cgm4 do not show a significant difference in the number of stomata or stomatal patterning compared to the wild type (Col-0). However, we found that the over-expression line of CGM4 was highly susceptible to drought stress exposure and stomata remained open even after abscisic acid (ABA) treatment. Furthermore, the cgm3cgm4 double knock out mutant showed significantly higher resistance to drought compared to wild type (Col-0) and the CGM4 over expression line. The cgm3cgm4 lines also showed significantly more closed stomata (with and without ABA treatment). We speculate that despite their apparent homology, different GDSL lipases are involved at specific time points during stomatal development in currently unknown mechanisms and that disturbance of their differential expression leads to subtle malfunction in mature stomata only visible under stress conditions.

Keywords: GDSL-Lipase esterase, tomata-development, meristemoid cells, drought stress

P35

Molecular dynamics after pollination revealed by SNP-based RNAseq analysis

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With the aim to unravel the molecular dynamics that occurs both at the female organ surface and in the pollen grain following pollination, we carried out a transcriptomic analysis. To distinguish stigma and pollen derived transcripts, we took advantage of the single nucleotide polymorphisms existing between two *Arabidopsis thaliana* accessions, one used as female and the other as male. We constructed data analysis pipeline and succeeded in distinguishing 80 % of mRNAs according to their female or male origin, and drew up a catalog of genes whose expression is rapidly modified after pollen-stigma interaction. Global analysis of our data reveals that specific signaling cascades involved in plant response to pathogen attack are induced on the female side after pollination. This strongly suggests that plant responses to pollen and pathogens are mediated by evolutionary conserved mechanisms, as a generic response to invaders.

Keywords: cell-cell communication, stress response, signaling, pollen-stigma interaction, RNA-seq

P36

Identification and transcriptome analysis of pollen number controlling gene, RDP1

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Pollen number is critical for reproductive success and varies greatly between and within species. We identified pollen number controlling gene, Reduced Pollen Number 1 (RDP1), by GWAS using 144 *Arabidopsis thaliana* accessions. CRISPR/Cas9 generated RDP1 null mutants produced about half the number of pollen grains of the wild type. Expression analysis of RDP1 showed RDP1 express very young pollen stage. RDP1 is homolog of yeast Mrt4 and it works ribosome and transcriptome analysis showed many ribosomal related genes were up-regulated in rdp1 mutant. These results suggest RDP1 works as ribosomal protein and RDP1-related protein synthesis is more important in the pollen cell generation.

Keywords: pollen, GWAS, RNA-seq, CRISPR

P37

Modeling plant mechanics & growth with MorphoDynamX

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The increasing interest for mechanical properties of living tissues and their interaction with gene expression to shape organ formations and functionalities as well as the advance in experimental and imaging techniques poses new challenges in terms of results interpretation and creation of predictive models. MorphoDynamX is an integrated modeling framework where experimental data from imaging can be digitalized, analyzed (MorphoGraphX) and used as starting template for mechanical simulation of growth. With MorphoDynamX different growth hypotheses, including the combined effect of genetic expression and stress/strain feedback models, can be tested to understand the underlying mechanisms of organs/tissues formation.

Keywords: morphodynamix, biomechanics, growth, feedback, simulation

P38

Keeping track of starch granule development with multi-modal imaging

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Starch is a key product of carbon fixation that results from the polymerisation of glucose into insoluble and semi-crystalline granules. Starch is found in chloroplasts under the form of transient starch and in amyloplasts for long storage. Granules are first initiated, then expanded by appending chains of glucose, and eventually degraded. Although glucan elongation has been extensively studied, the mechanisms that control initiation remain enigmatic. Here we use the model plant *Arabidopsis thaliana* to investigate initiation patterns as well as the subsequent expansion during the day. Our strategy to trace back the granule development consists in pulsing air containing isotopically stable 13C-carbon dioxide for a given period of time, by computing the enrichment in the given stable isotope. At the end of the pulse, we fix, dehydrate and embed in epoxy resins leaf samples. We then image the regions of interest using transmission electron microscopy. We finally scan the same surface with an ion beam which fractionate the sample's surface, eventually desorbing ions of selected elements. A coupled mass spectrometer diffracts the ions and count them. The output is an array that contains the counts of each of the ions detected. These data collected with this method are integrated with other imaging technologies and genetic approaches to further our understanding of granule development.

Keywords: starch, *Arabidopsis*, nanoSIMS, TEM, granule

P39

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. An assay was designed to understand differential effects of endogenous and exogenous auxins on lateral root formation. Furthermore, a transcriptomics approach will help to identify candidate genes, which are orchestrating lateral root initiation and development.

P40

Discrete genetic elements drive diversity effects in conspecific plant communities

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Ecological experiments have frequently found a positive relationship between the biological diversity and the productivity of communities. Such effects have, however, neither been fully explained at the functional level, nor have they received enough attention from a breeding perspective. I have examined positive genetic diversity effects in conspecific communities consisting of different *Arabidopsis thaliana* accessions, for which genetic mapping populations are available. Results from a quantitative genetic approach indicate that these effects can be driven by allelic variation at discrete loci. The mendelization and molecular cloning of such discrete genetic elements is currently in progress. I have further fine-mapped one locus with major effects at which allelic diversity between two accessions drives overyielding in genotype mixtures to a one centimorgan interval. This has led to the identification of a possible candidate DNA polymorphism in a gene known to mediate in a trade-off between growth and defense, and a functional validation of this is ongoing. The work provides proof-of-principle that a genetic approach can help identify genes, traits and mechanisms that underlie niche differentiation in conspecific plant communities, and (by the use of population genetics) allow for the study of evolutionary forces shaping biodiversity effects. It furthermore shows that a community-breeding approach that selects for allelic diversity at field-level is in reach of current possibilities.

P41

Elucidating the molecular basis for the role of protein degradation in defence response

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Protein levels inside cells are partly regulated by specific protein degradation with the Ubiquitin-26S proteasome system (UPS). This system is large and complex, occupying nearly 6% of the coding capacity of the *A. thaliana* genome. The largest class of proteins involved in the UPS are the E3 ubiquitin ligases, which are responsible for mediating the transfer of the activated ubiquitin to the respective target proteins. The plant U-box E3s (PUBs) are a class of E3s that are considered to be important in mediating plant-specific processes. Several of the PUBs in *Arabidopsis* have been associated with a role in defense response, but few target proteins could be identified so far. Using distinct pub mutant *Arabidopsis* lines coupled with protein-protein interaction detection assays (Tap-Tag & BioID) and methods to identify the sites of ubiquitylation we aim to elucidate the molecular basis of different PUBs in defense response, and to gain novel insight into this biological process.

P42

The influence of snow cover duration on alpine plant phenology

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In the alpine region, the winter is long and plants adopt different phenological strategies to cope with the short growing season. While they have to synchronize flowering to ensure reproductive success, they also have to prevent freezing damages early and late in the season. Snow insulates the soil and allows for soil processes to continue over winter, determines the length of the growing season and protects plants from freezing. In a manual snow addition and removal experiment at 2500 m a.s.l., we explored the consequences of altered snowmelt dates on phenology of different plant species in a typical alpine grassland. We revealed different phenological strategies. Species like *Carex curvula* flower only few days after snowmelt and show an opportunistic flowering phenology. Thermal thresholds also trigger *Potentilla aurea* to flower, but these species take more time to develop. Few species, as for example *Poa alpina*, are at least partly controlled by photoperiod. We could show that the altered timing of snowmelt reveals the different strategies of alpine plant species to cope with short growing seasons.

Keywords: phenology, ecology, physiology, Alpine, grassland

P43

Non-parallel molecular evolution of the moth pollination syndrome in the sister genera *Calibrachoa* and *Petunia*

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Astonishing floral diversity is one of the most interesting aspects of the rapid radiation of the angiosperms. Studies performed in many species support the hypothesis that interactions with pollinators drive this floral diversity. Evolutionary shifts from one pollinator to another often involve changes in either type or quantity of produced floral pigments, so-called pollination syndrome. Similar shifts in pollination syndromes have happened in many different taxa, which raises the question of whether such convergence also holds at the molecular level. In *Petunia*, causal agent of the difference between the purple bee-pollinated *P. integrifolia* and the white hawkmoth-pollinated *P. axillaris* is the gene AN2, inactivation of which promotes the loss of pigmentation in *P. axillaris.* A similar pollinator shift has evolved independently in *Calibrachoa*, the sister genus to *Petunia. C. parviflora* with pink scentless, adapted to be bee-pollinated flowers and *C. pygmaea* with a moth pollination syndrome characterized by a white corolla and strong scent emission at dusk together create an attractive system for comparative study of the molecular basis of convergent evolution, at the same time being phylogenetically close to *Petunia* for easier homolog determination. By generating and analysing transcriptome data for *C. parviflora* and *C. pygmaea* and their artificial F1 hybrid, surprisingly, we find that the convergent evolution of moth pollination employed a divergent route at the molecular level. First, we found that AN2 gene is not likely to be responsible for the differences in flower colour between these two *Calibrachoa* species. Next, we found that unlike *Petunia*, *Calibrachoa* synthesize flower pigments from both anthocyanins and carotenoids class. Finally, very low expression levels of genes responsible for the anthocyanin vacuolar transport and vacuolar pH level maintenance in *C. pygmaea*, as well as the overall less expression of the late anthocyanin biosynthesis genes.

Keywords: anthocyanins, carotenoids, colour genes, pH, adaptation

P44

Regulation of guard cell starch metabolism by protein phosphorylation

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Stomata are pores on the leaf surface through which plants take up atmospheric CO_2 necessary for photosynthesis and lose water with transpiration. Each pore is delimited by a pair of guard cells. These specialized cells control the degree of aperture by changing their osmotic pressure with the intake of K+ ions and negative counterions, thereby promoting osmotic water flow. Guard cells contain large starch granules at the end of the night, which are quickly degraded upon illumination, a mechanism essential for stomatal opening. We showed that in *Arabidopsis* mutants impaired in blue light signaling guard cells do not degrade starch upon illumination. In particular, H+-ATPase activity is necessary for starch degradation to occur. We also discovered that guard cell starch degradation is mediated by BAM1, a beta-amylase protein expressed in chloroplasts. BAM1 is not involved in mesophyll starch degradation, a process that mainly relies on BAM3. Despite both proteins having the same catalytic domain, they acquired specific functions. In particular, BAM1 presents in its N-terminal domain a phosphorylation motif, which is recognized by GSK3/Shaggy-like kinases. We showed that the only chloroplastic member of this kinase family, AtK4, can phosphorylate BAM1 in vitro. Interestingly, AtK4 is specifically expressed in guard cells and loss-of-function atk4 mutant has abnormal starch accumulation specifically in guard cells. We are currently investigating the extent, nature and timing of BAM1 phosphorylation in *Arabidopsis* guard cells and the importance of this process for the regulation of guard cell starch metabolism.

Keywords: guard-cells, starch, blue-light, protein-phosphorylation

P45

Source-sink physiological changes and metabolic adjustments triggered by water deficit in two *Solanum lycopersicum* L. genotypes

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Drought stress limits the growth and yield of crops, affecting source-sink relationships. A dynamic signalling network, in which hormones, reactive oxygen species and sugars are mainly involved, is activated by plants to cope with water shortage. Tolerant plants have developed efficient adaptive mechanisms to establish new cell metabolism homeostasis avoiding and/or reducing permanent impairments triggered by drought. The Southern Italy tomato landrace Ciettaicale and the well-known tomato cultivar Moneymaker were compared evaluating their biometrical and metabolic responses to 20 days-water deficits under controlled growth chamber conditions. We evaluated *in vivo* chlorophyll a fluorescence, gas exchanges and leaf water potential, together with the analyses of non-structural carbohydrates in source and sink organs and with the hormonal and antioxidative responses. Leaf gas exchange measurements revealed higher water use efficiency (WUE) in Ciettaicale comparing to Moneymaker thanks to more efficient CO_2 assimilation capacity. Under drought Moneymaker showed lower starch content and, in parallel, higher sucrose level than Ciettaicale, both in leaves and roots. Moreover, elevated levels of hydrogen peroxide, lipid peroxidation and abscisic acid were recorded in Moneymaker leaf and root tissues. Changes in the antioxidant pool, including enzymes and compounds like ascorbate peroxidase, ascorbate and phenols, and compatible osmolites, such as proline, played a key role to counteract redox and osmotic pressure in both genotypes. Overall, while Moneymaker showed a survival strategy leading to the accumulation of metabolites to maintain more negative leaf water potential but at same time effecting the source-sink balance, Ciettaicale increased WUE and maintained carbon translocation from source to sink supporting the investment in root metabolism and growth to avoid drought-related osmotic pressure.

Keywords: water deficit, tomato, source-sink balance, carbon partitioning

P46

Does microRNA regulate axillary meristem development in *Arabidopsis*?

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Plant organs are generating throughout plant lifetime due to a continuous activity of the shoot apical meristem. During the vegetative phase in *Arabidopsis*, leaves are formed at the apical meristem, subsequently axillary meristems develop in leaf axils and give rise to lateral branches. However, after the transition to flowering the axillary meristems are formed soon after leaf initiation. This might suggest different mechanisms involved in axillary meristem development during shoot ontogeny. On the other hand, a detailed analysis of gene expression at the shoot apical meristem showed, that the position of future axillary meristem is specified concomitant with the initiation of leaves during both vegetative and reproductive phases (Burian et al. 2016, Curr Biol). Thus, the earliest stages in axillary meristem development are similar during shoot ontogeny, and there is only different timing in the later stages. We aim at finding signals that would be involved in a delay in the formation of axillary meristems relative to leaf initiation during the vegetative phase in *Arabidopsis*. We follow morphogenetic processes and gene expression patterns at the apical meristem during shoot ontogeny using high-resolution time-lapse imaging. In particular, we focus at miRNA165/166, which determinate the adaxial site of leaves and regulate the expression of HD-ZIP III genes that are crucial for meristem establishment. We found that during the vegetative phase miR165A is expressed not only at the abaxial leaf primordia (as other miR156 and 166) but also in the position of the future axillary meristem. The latter expression disappears during the transition to flowering, when the formation of axillary meristems is not delayed. Accordingly, we propose that the timing of axillary meristem development in *Arabidopsis* might be regulated by miRNA165A.

Keywords: meristem, microRNA, *Arabidopsis*