BOTANIKER TAGUNG 2013

ABSTRACT BOOK

BOTANIKERTAGUNG 2013 - ABSTRACT BOOK
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BOTANIKERTAGUNG 2013 ABSTRACT BOOK

29.September - 04. October - Tübingen - Germany

CONTENT

ABSTRACTS OF PLENARY TALKS

PLENARY TALK I

Structure, function(s), and evolution of the bacterial root microbiota

Paul Schulze-Lefert[1], Klaus Schläppi[1], Davide Bulgarelli[1], Emiel ver Loren van Themaat[1], Nina Dombrowski[1], Matthias Rott[1], Yang Bai[1]

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Plants host distinct bacterial communities on and inside various plant organs. We show that roots of *Arabidopsis thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetes and Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. Plant cell wall features provide a sufficient cue for the assembly of ~40% of the Arabidopsis bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. A comparison of the bacterial root microbiota of *A. thaliana* with *A. lyrata*, *A. halleri* and *Cardamine hirsuta*, grown under controlled environmental conditions or collected from natural habitats, revealed the existence of an evolutionarily conserved core microbiota with species-specific footprints, suggesting this core is robust against environmental changes. We have isolated > 60% of the root microbiota members from *A. thaliana* as pure bacterial cultures, permitting whole-genome sequencing and *in silico* analysis of the root microbiome. We have begun to test synthetic rhizobacterial communities under laboratory conditions to explore microbiota functions in plant growth promotion and plant health.

PLENARY TALK II

Phytochrome Interacting Factors (PIFs) regulate plant growth in a changing light environment.

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Plants such as *Arabidopsis thaliana* respond to foliar shade and neighbor proximity by elongation growth and leaf hyponasty to secure access to unfiltered sunlight. The phytochrome B (phyB) photoreceptor is the major light sensor mediating these adaptive responses. The shade avoidance response (SAR) involves phyB directly controlling protein abundance of Phytochrome Interacting Factors 4 and 5 (PIF4 and PIF5). PIF4 and PIF5 rapidly promote growth via auxin-mediated processes and simultaneously turn on HFR1, a negative regulator of SAR (1-3). Using a computational modeling approach to study the SAR regulatory network, we identify and experimentally validate a novel role for HFR1. Moreover, we developed a novel phenotyping platform allowing us to analyze leaf growth and positioning with great spatial and temporal resolution (4). This allows to test whether the gene regulatory network controlling young seedling development operates in a similar manner later in development.

1) Lorrain et al., *Plant J* 2008, **53:**312-23.

2) Hornitschek et al., *Embo J* 2009, **28:**3893-902.

3) Hornitschek et al., *Plant J* 2012, **71:**699-711.

4) Dornbusch et al., *Functional Plant Biology* 2012, **39:**860-69.

PLENARY TALK III

Plants, pipes, and ancient dreams: Using metabolomics for chemotaxonomic investigations

Oliver Fiehn[1], Dominique Ardura[1], Mine Palazoglu[1], Jelmer Eerkens[1], Shannon Tushingham[1]

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Metabolomics technologies have greatly advanced over the past 15 years. We here present a short overview over current technology platforms, libraries and data processing and statistical methods used in metabolomics. We applied these methods in a chemotaxonomic investigation of plants native to California to answer the question, what type of plants did members of tribal Americans in the Pacific Northwest Region of California smoke in their ceremonial pipes before European settlers arrived? A range of ethnographically important California plants were compared by time-of-flight based metabolomics both as plant extracts and after smoking dried specimen in experimental clay pipes. Hundreds of different chromatographic peaks were extracted and annotated by spectral comparisons. Quantitative profiles were subsequently used to cluster plant extracts to yield chemotaxonomic relationships among these plants and to find novel compounds distinguishing closely related plants. Archaeological residues extracted from seventeen clay and steatite (soapstone) pipe fragments revealed a specific tropane alkaloid found in a subterranean plank house dating to AD 863.

S Tushingham, D Ardura, J Eerkens, M Palazoglu, S Shahbaz, O Fiehn (2013) Journal of Archaeological Science, 40: 1397-1407

PLENARY TALK IV

Seasonal control of potato storage organ formation: the *Solanaceae* photoperiodic pathway.

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Potato tubers differentiate from underground stems or stolons. In nature, these organs are formed as winter approaches. Plants use seasonal changes in day length and temperature as the informational cue that triggers formation of these organs. Day length is perceived by the leaves. If day length duration is shorter than a minimal threshold (critical photoperiod), a mobile signal or "*tuberigen*" is produced in the leaf minor veins and transported via the phloem to the underground stolons, where it promotes tuber transition. Work by our group has shown that this mobile signal is encoded by a member of the *FLOWERING LOCUS T* (FT) gene family, the *SP6A* gene. In *Solanaceae* this gene family has undergone preferential expansion, with 5 members identified in the potato genome. Interestingly, the transcriptional factor CONSTANS does not regulate expression of the SP6A mobile signal, but is involved at activation of an additional member of the gene family, the *SP5G* gene. *SP6A* is actually upregulated in phloem cells only when the negative SP5G regulator is no longer expressed. Specific activation of the SP6A mobile signal in short days thus relies in the endogenous clock and light dependent stabilization of the CONSTANS factor, that drives expression of the SP5G negative regulator in LDs. The nature of the factor responsible to activate *SP6A* expression in SDs is still unknown. Search for SP5G interactors is now underway to gain insights into this important regulator.

PLENARY TALK V

Enhancing photosynthesis and carbon fixation

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In the last century, scientists uncovered much of the fundamental basis for life culminating in the ability to decipher the genome sequence of all organisms on the earth. Computational tools and technological developments together with molecular biology have led to a detailed knowledge of much of the inner workings of cells and organisms. Much as organic chemistry and microchips fueled the industry of the past century, we are poised to use this vast knowledge to logically engineer biology and launch a new industrial revolution that could impact livability of all. We thus seek to make the engineering of biology faster and more predictable - creating a synthetic biology. We use predictable engineering to alter the productivity of photosynthetic organisms. Some of our experiments use the simpler autotrophic cyanobacteria. We also move carbon fixation to organisms that do not normally fix carbon and increase the ability of plants to fix carbon and increase their biomass. In one instance, we have reconstructed the carboxysome and discovered how it replicates. We have re-routed carbon metabolism in autotrophs such that they increase their photosynthetic capacity. We believe this may lead to a general strategy to optimize photosynthesis.

PLENARY TALK VI

Chemical Biology Reveals Insights into Endomembrane Trafficking

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Endomembrane trafficking is essential for coordinated growth and development in plants and response to environment. In particular, key plasma membrane proteins such as PIN auxin transporters and the BRI1 brassinosteroid receptor translocate between the endosomes and plasma membrane. The discovery of the important roles of the endomembrane system in hormone signaling in the last decade has contributed greatly to the understanding of signal transduction pathways and their integration during plant growth and development. Due to genetic redundancy and the highly dynamic properties of the endomembrane system, it is challenging to use traditional knock-out mutants alone to study trafficking. Chemical genomics provide a valuable tool in solving these problems by the rapid action, specificity and reversibility of small molecules. A very successful example is Brefeldin A which has been widely used in to study trafficking and signaling. Through a previously published large-scale chemical library screening approach, we identified groups of small molecules that affect trafficking of PIN auxin transporters and other plasma membrane proteins in Arabidopsis. We are currently combining biochemical and proteomic approaches with genetic screening to identify the target of several bioactive compounds. Our latest results will be discussed in dissecting trafficking via endosomes in Arabidopsis.

PLENARY TALK VII

The Hidden Signposts of Development

Enrico Coen[1]

[1]John Innes, Norwich, United Kingdom

Development involves highly oriented cell behaviours, such as anisotropic growth and asymmetric cell divisions. It is unclear how these orientations are specified and how they lead to particular cellular or tissue outcomes. We have been addressing this problem using a combination of genetic, morphological, computational and imaging. The results provide new insights into how genes interact to specify orientations of growth and division, leading to particular shapes. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development.

PLENARY TALK VIII

Transgenic plastids as expression factories in biotechnology

Ralph Bock[1]

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The plastid genome represents an attractive target of genetic engineering in crop plants. Plastid transgenes often give very high expression levels, can be stacked in operons and are largely excluded from pollen transmission, thus providing increased biosafety of transgenic crops. Recent research has greatly expanded our toolbox for plastid genome engineering and many new proof-of-principle applications have highlighted the enormous potential of the transplastomic technology in both crop improvement and the development of plants as bioreactors for the sustainable and cost-effective production of biopharmaceuticals, enzymes and raw materials for the chemical industry. In my talk, I will describe recent technological advances with plastid transformation in seed plants and summarize progress with harnessing the potential of plastid genetic engineering in biotechnology. Selected applications in two key areas of plant biotechnology will be discussed: metabolic pathway engineering and molecular farming.

ABSTRACTS OF ORAL SESSIONS

1.1 TALK 1 – SYMPOSIA 1 – 14:00–14:30

Protein ubiquitination in light-controlled anthocyanin biosynthesis and photomorphogenesis

Alexander Maier[1], Sebastian Rolauffs[1], Andrea Schrader[1], Leonie Kokkelink[1], Joachim Uhrig[1], Martin Hülskamp[1], **Ute Hoecker**[1]

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Anthocyanins accumulate only in light-grown and not in dark-grown Arabidopsis plants. Repression of anthocyanin accumulation in darkness requires the COP1/SPA ubiquitin ligase. Here, we show that COP1 and SPA proteins interact with the MYB transcription factors PAP1 and PAP2, two members of a small protein family that is required for anthocyanin accumulation. PAP1 and PAP2 proteins are degraded in darkness and this degradation is dependent on the proteasome and COP1. This suggests that PAP1 and PAP2 are novel substrates of the COP1/SPA ubiquitin ligase. We also investigated the roles of the COP1/SPA complex in seedling and adult facets of the shade avoidance response. We show that *COP1* and the four *SPA* genes are essential for hypocotyl and leaf petiole elongation in response to low R:FR, in a fashion that involves HFR1 but not HY5. In contrast, the acceleration of flowering in response to a low R:FR ratio is normal in *cop1* and *spa* mutants. Taken together, our results indicate that COP1/SPA activity, via HFR1, is required for shade-induced increase in auxin biosynthesis, leading to enhanced cell elongation.

1.1 TALK 2 – SYMPOSIA 1 – 14:30–14:50

Characterization of an animal-like cryptochrome in *Chlamydomonas reinhardtii*

Benedikt Beel[1], Christin Gebauer[1], Sandra Kuenzel[1], Tilman Kottke[2], Maria Mittag[1]

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[2]Physical and Biophysical Chemistry, Department of Chemistry, Bielefeld University, Bielefeld, Germany

Cryptochromes are flavoproteins that are known as blue light photoreceptors in many organisms including plants. We have investigated a cryptochrome from the green alga *C. reinhardtii* with sequence homology to animal cryptochromes and (6-4) photolyases. In response to blue but also yellow and red light exposure, this animal-like cryptochrome (aCRY) influences light-dependent transcription of various genes encoding proteins involved in chlorophyll and carotenoid biosynthesis, light harvesting complexes, nitrogen metabolism, and the circadian clock. The *in vitro* spectroscopy and the *in vivo* gene expression data indicate that the aCRY neutral radical state acts as a sensory blue and red light receptor (1). It is known that the circadian clock is entrained by environmental stimuli such as the daily light-dark cycle, involving different photoreceptors. In *C. reinhardtii*, red light resets the phase of the circadian rhythm of phototaxis (2). Currently, we are investigating if aCRY is involved in this process. First data indicate differences in the phase response curve in wild type and the *acry* mutant strain.

(1) Beel et al., Plant Cell, (2012) 24: 2992-3008; (2) Kondo et al., Plant Physiol. (1991) 95, 197-205

1.1 TALK 3 – SYMPOSIA 1 – 14:50–15:10

Downy mildew effector protein HaRxL106 associates with Arabidopsis RADICAL INDUCED CELL DEATH1 and suppresses defence and light signalling

Lennart Wirthmueller1, Georgina Fabro1, Shuta Asai1, Ghanasyam Rallapalli1, Michael Wrzaczek[2], Stefan Kircher[3], Jaakko Kangasjärvi[2], Mark Banfield[4], Jonathan Jones[1]

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Oomycete pathogens overcome plant immunity by translocating effector proteins into host cells that interfere with plant defence. *Hyaloperonospora arabidopsidis* (*Hpa*) effector HaRxL106 localises to the host cell nucleus and interacts with the putative ADP-ribosyltransferase RCD1. RCD1 binds several transcription factors involved in light and defence signalling. HaRxL106 over-expression in Arabidopsis induces constitutive shade avoidance. The transcriptome profile of HaRxL106-expressing plants resembles the one induced by shade, including suppression of JA- and SA-dependent defence genes. *rcd1* mutants are more light sensitive and thus show the opposite phenotype of HaRxL106-expressing lines. The C-terminal 58 amino acids of HaRxL106 are sufficient and required to induce both shade avoidance and susceptibility to *Hpa*. This 58 amino acid peptide binds to an N-terminal RCD1 fragment that includes its putative catalytic PARP domain. We propose that HaRxL106 modifies the activity of RCD1 to manipulate light and defence signalling of the host. We also present the crystal structure of the RCD1 PARP domain and discuss if RCD1 is an ADP-ribosyl-transferase.

1.1 TALK 4 – SYMPOSIA 1 – 15:10–15:30

MIDGET unravels an archaeal fingerprint in endoreduplication and light signalling of *Arabidopsis thaliana*

Andrea Schrader[1], Bastian Welter[1], Viktor Kirik[2], Ute Höcker[1], Martin Hülskamp[1], Joachim F. Uhrig[3]

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[2]Illinois State University, Normal, United States
[3]Georg-August-Universität, Göttingen, Germany

Topological constraints of DNA double-strands are resolved by topoisomerases like the archaeal topoisomerase VI. Its homolog in *Arabidopsis thaliana* (*A. thaliana*) is essential for the progression of endoreduplication cycles (endocycles) (Corbett and Berger, 2003). We identified MIDGET (MID) as a component of the *A. thaliana* topoisomerase VI (AT-TOPOVI) complex (Kirik et al., 2007).On the one hand, we provide evidence for its involvement in the checkpoint control of endocycle and DNA damage response (Kirik et al., 2007). On the other hand, physical and genetic interaction of MID with CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) positions AT-TOPOVI in the context of light signalling (Schrader et al., 2013a).Light is tightly regulating the plant`s life cycle. Under unfavourable conditions, photomorphogenesis and flower induction are suppressed by the COP1/SUPPRESSOR OF PHYTOCHROME A (SPA) complex. This complex participates in the ubiquitylation and subsequent degradation of transcription factors. We have shown that MID is needed for the COP1-mediated suppression of photomorphogenesis and COP1/SPA controlled flowering under short day conditions (Schrader et al., 2013a; b (accepted)). Taken together, MID provides a functional connection between AT-TOPOVI dependent regulation of endocycles and COP1-controlled development.

1.2 TALK 1 – SYMPOSIA 9 – 10:30–11:00

Lateral root organ initiation rephases the circadian clock in *Arabidopsis thaliana*

Malcolm Bennett[1]

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The endogenous circadian clock enables organisms to adapt to diurnal environmental changes and has been identified in all domains of life. However, how the clock synchronises growth processes across multiple cells and tissues is poorly understood. Here we describe how the circadian clock is employed to coordinate responses between multiple tissues during lateral root organogenesis and emergence. In the model plant, *Arabidopsis thaliana*, lateral roots originate from a group of stem cells deep within the primary root, necessitating that the new organ push through the overlying tissues. Induction of new lateral root organs resets the circadian clock in a phase independent of the primary root or shoot. Disrupting clock function impaired lateral root emergence, but not by perturbing the clock in just the new lateral root organ. We conclude that roots employ the circadian clock to coordinate responses in tissues overlying new organs to facilitate their emergence.

1.2 TALK 2 – SYMPOSIA 9 – 11:00–11:20

A receptor protein links cell wall surveillance with hormone signalling

Sebastian Wolf[1], Dieuwertje van der Does[2], Friederike Ladwig[3], Carsten Sticht[4], Klaus Harter[3], Cyril Zipfel[2], Herman Hoefte[5]

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[4]ZMF, MAnnheim, Germany
[5]INRA, Versailles, France

The growth and shape of plants is governed by the properties of their cell walls, which dynamically adapt to internal and external cues. Therefore, the cell wall is under constant surveillance to relay feedback information to the cell interior [1]. However, very little is known about how cell wall signalling is integrated with intracellular growth regulation. Recently, we have shown that interference with wall modification triggers activation of the brassinosteroid (BR) signalling pathway, which in turn orchestrates a compensatory response involving cell wall remodelling [2]. Here, we show through the use of a forward genetics screen that CNU2, a receptor-like protein, is essential for cell wall-induced activation of the BR pathway. Furthermore, we demonstrate that CNU2 directly interacts with the regulatory receptor-like kinase BAK1, thus representing the convergence point of cell wall and brassinosteroid signalling. Conditional, CNU2-mediated signalling activation is required for normal development and stress responses, requires functional brassinosteroid receptor BRI1, but is partially independent of the hormone ligand.

[1] S. Wolf et al. (2012) Annu Rev Plant Biol 63:381-407

[2] S. Wolf et al. (2012) Curr. Biol., 22 (18), 1732-7.

1.2 TALK 3 – SYMPOSIA 9 – 11:20–11:40

ROCK1 encodes a novel nucleotide sugar transporter influencing the cellular cytokinin homeostasis

Michael Niemann[1], Isabel Bartrina[1], Angel Ashikov[2], Hans Bakker[2], Thomas Schmülling[1], **Tomas Werner**[1]

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[2]Hannover Medical School, Department of Cellular Chemistry, Hannover, Germany

The plant hormone cytokinin is an essential regulator of many physiological and developmental processes in plants and its cellular concentration must be precisely regulated. Cytokinin deficiency causes complex phenotypic changes such as retarded shoot and enhanced root growth. In order to uncover new functional elements of the cytokinin homeostatic control system, we screened for suppressor alleles that reverse the retarded shoot development of cytokinin-deficient *35S:CKX1* transgenic *Arabidopsis* plants. The recessive mutant allele *rock1* (*repressor of cytokinin deficiency 1*) was identified to negatively regulate several CKX isoforms, hence influencing strongly the cytokinin responses. A map-based cloning of the corresponding gene and functional studies of the encoded protein revealed that ROCK1 represents an as yet uncharacterized nucleotide-sugar transporter localized to the endoplasmic reticulum. Study on substrate specificity of this transporter will be presented and its possible physiological function discussed.

1.2 TALK 4 – SYMPOSIA 9 – 11:40–12:00

Insect-induced jasmonates and carnivorous plants

Axel Mithöfer[1], Yoko Nakamura[1], Michael Reichelt[2]

[1]Max-Planck-Institute for Chemical Ecology, Bioorganic Chemistry, Jena, Germany
[2]Max-Planck-Institute for Chemical Ecology, Biochemistry, Jena, Germany

Carnivorous plants of the worldwide occurring genus Drosera catch their prey by employing adhesive traps, which present sticky polysaccharide mucilage. The glue is produced by stalked glands, tentacles, on the leaves surface. The glands also secrete enzymes to digest captured prey and subsequently absorb generated nutrients. The whole process is elicited by mechanical and chemical stimuli from the prey that induce electric potentials in the glands, which moves towards the basis of the tentacles in the epidermal layer of the leaf. Beside this fast tentacle movement a second, slower movement of the whole leaf starts to enclose the caught prey and to form a so-called outer stomach. Although Darwin already described this leaf bending phenomenon, the prey-induced endogenous plant signals that direct this movement remained to be identified. Using fruit flies, *Drosophila melanogaster*, and the sundew species *Drosera capensis*, the induction of 'outer stomach' formation was analysed. The kinetics of leaf bending as well as a novel role for jasmonates will be described. Based on the results presented, the evolution of carnivory in *Drosera* will be discussed.

Nakamura et al., Proceedings of the Royal Society B: Biological Sciences, 280: 20130228

1.3 TALK 1 – SYMPOSIA 4 – 14:00–14:30

Epigenetic plasticity under heat stres

Ortrun Mittelsten Scheid[1], Jasmin Bassler[1], Laura M. Bayer[1], Vanja V. Cavrak[1], Nina Daubel[1], Ruben Gutzat[1], Nicole Lettner[1], Ales Pecinka[2]

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Stress can trigger genetic changes, but there is growing evidence that external factors can also modulate epigenetic regulation. Plants are suitable organisms to test the degree of epigenetic plasticity and the participating molecular processes, due to an impressive potential to adapt to various conditions and the diversification of epigenetic regulators.Heat stress in plants induces signalling cascades that rapidly lead to the production of heat-protective factors. Enduring heat stress conditions result in activation of additional sets of genes, including repetitive elements, which are transcriptionally silenced at ambient temperatures or after short heat stress exposure. In contrast to other conditions that interfere with gene silencing, DNA methylation and histone modification are only marginally affected by extended heat stress, while nucleosome occupancy and chromatin organization within the nucleus undergo substantial changes. This can modify the accessibility of DNA for transcription, but also for DNA repair, recombination or other genetic changes. Whereas resulting DNA sequence modification are naturally heritable, most epigenetic changes are transient and reversible. However, stable inheritance of solely epigenetic changes through the germ line is theoretically possible and a matter of current research and debate.

Pecinka et al. (2010) Plant Cell 22: 3118-3129; Pecinka and Mittelsten Scheid (2012) Plant Cell Physiol 53: 801-808

1.3 TALK 2 – SYMPOSIA 4 – 14:30–14:50

Improved freezing tolerance by *pr-10a* overexpression

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A transgenic potato cell line was established by homologous overexpression of the *pr-10a* gene under the control of a mannopine synthase promoter. It showed improved growth and altered proline and glutathione metabolism under salt and osmotic stress compared to the wild type. As cryopreservation exposes the cells to this kind of stresses, we investigated whether *pr-10a* overexpression also improved freezing tolerance, using a controlled rate freezing approach (osmotic preculture, cryoprotection and programmed freezing). Varying the concentration of the preculture osmotic the transgenic culture showed always higher survival rates than the wild type, but unexpectedly the same optimum (0,6M) curve, which was only shifted to higher survival. Measurement of *pr-10a* transcript levels by q-PCR demonstrated, that the genetic modification did not affect the expression of the endogenous *pr-10a* gene, but that the transgenic cell line exhibited a higher total transcript level of *pr-10a* under all preculture conditions. Variation of pretreatment duration revealed that the maximum of total *pr-10a* transcript level occurred after 12 hours, but higher survival rates of the transgenic cell culture were detected only after more than 24 hours of pretreatment. The results indicate a modulating rather than a direct effect of the PR-10a protein on freezing tolerance.

1.3 TALK 3 – SYMPOSIA 4 – 14:50–15:10

Dissecting the Role of FIERY1 in different Plant Cell Compartments

Natallia Ashykhmina[1], Eduard Hofsetz[1], Ulf-Ingo Fluegge[1], Tamara Gigolashvili[1]

[1]University of Cologne, Department of Botany II, Cologne, Germany

Plant cell is the most highly compartmentalized eukaryotic cell due to the presence of plastids. Plastids and mitochondria are dependent on the permanent flow of information from nuclei, known as an anterograde signalling, but they also have their own genetic information consisting of 128 and 57 genes respectively. Therefore, the plant cell requires tightly organized retrograde signalling - the information sent from chloroplasts and mitochondria back to the nuclei. 3´-Phosphoadenosine 5´-phosphate (PAP) has been recently proposed as mobile signal molecule in retrograde signalling. PAP, produced from 3´-phosphoadenosine 5´-phosphosulfate (PAPS) in cytosol, can trigger posttranscriptional gene silencing (PTGS) via binding to XRNs. FIERY1 (FRY1) is a 3'(2') 5'-bisphosphate nucleotidase, which dephosphorylates the PAP to AMP both in plastids and mitochondria. Surprisingly, expression of FRY1 protein in the cytosol and nuclei was able to complement the *fry1* phenotype. To dissect the role of FRY1 protein in chloroplasts, mitochondria, nuclei and cytosol, we have attempted the complementation of a *fry1* mutant with FRY1 protein specifically directed to these cell compartments. Details on the complementation of a *fry1* mutant with FRY constructs directing its expression in to the cytosol, nuclei, chloroplast and mitochondria will be presented and discussed.

1.3 TALK 4 – SYMPOSIA 4 – 15:10–15:30

The elevated level of H2O2 is a consequence of low availability of sulfur to shoot under drought stress in *Zea mays*

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Drought tolerance is the most important trait in maize, since limitation of water supply limits yield at most. The enhanced production of ROS during drought requires an increased GSH production for the efficient detoxification of ROS, thus the regulation of sulfur assimilation during drought is vital due to the dependency of GSH synthesis on the sulfur assimilation pathway. Maize seedlings exposed to drought for 10 and 12 days were severely affected in leaf biomass due to a decrease in plant water content and caused elevated levels of H2O2. The drought-induced increase in the ROS formation altered the redox state of GSH pool towards a more oxidized state and indicated oxidative stress in leaves of drought-treated plants compared to control. Moreover, induction of *GR* transcription and activity in leaves under drought imply an important role of GR in ROS detoxification and maintaining reduced GSH during drought. The up-regulation in the *Sultr1;1* and *Sultr4;1* and a decrease in the steady state levels of sulfate most likely indicate sulfur-starved situation in leaves during drought. A reduction in the incorporation of 35S into cysteine and GSH suggests that drought limits the availability of sulfate to shoot, thus causing lower flux through the sulfur assimilation pathway into GSH.This clearly indicates that drought limits the availability of sulfate to shoot, thereby causing the down regulation of sulfur assimilation pathway and ultimately elevated levels of H2O2 in leaves.

1.4 TALK 1 – SYMPOSIA 5 – 16:00–16:30

Environmental adaptation in Arabidopsis halleri

Ute Kraemer[1], Ricardo Stein[1], Juergen Kroymann[2], Marc Hanikenne[3], Stephan Hoereth[4], Vasantika Singh[1], Christa Lanz[5], Xi Wang[5], Detlef Weigel[5], Stephan Clemens[4]

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As a typical representative of around 500 known metal hyperaccumulator taxa, *Arabidopsis halleri* accumulates exceptionally high leaf metal concentrations of up to > 10,000 µg g-1 Zn and > 100 µg g-1 Cd in dry biomass, thereby contrasting with its sister species *A. lyrata*, and with *A. thaliana*. Earlier transcriptomics studies identified around 30 metal homeostasis candidate genes based on elevated transcript abundance in highly metal-tolerant accessions of *A. halleri* when compared to *A. thaliana*. Among these, three tandem copies of *HEAVY METAL ATPase4* (*HMA4*), which encode a P1B-type Zn2+/Cd2+-pump acting in metal export from specific cells, make decisive contributions to both metal hyperaccumulation and hypertolerance. Alterations in promoter sequences further contribute to high *HMA4* transcript abundance in *A. halleri*, with evidence for positive selection. In order to begin addressing within-species genetic diversity and local adaptation in *A. halleri*, we determined metal concentrations of leaves and rhizosphere soils, alongside other individual- and site-specific parameters, at more than 100 collection sites across Europe. Assays of metal accumulation under controlled conditions and sequencing-based genotyping are in progress. Our results pinpoint *A. halleri* as an exquisite satellite species for addressing the genetic basis of plant adaptation to the environment.

1.4 TALK 2 – SYMPOSIA 5 – 16:30–16:50

PIC-NIC in plastid envelopes - towards decoding chloroplast metal-translocon complexes

Katrin Philippar[1], Roland Stübe[1], Daniela Duy[1]

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Chloroplast transport of transition metals like Cu, Mn and Fe is essential, since they are central to e-- transport in photosynthesis. However, metals are toxic when present in their highly reactive, free ionic form. Thus, plastid metal homeostasis and transport has to be tightly balanced and is crucial for plant development. The protein PIC1 functions in Fe-uptake across the inner chloroplast envelope (Duy et al. 2007; 2011). Chlorotic and dwarfish *pic1* k.o. mutants are reminiscent of Fe-deficiency symptoms, show severely impaired plastid development and increased ferritin Fe-storage proteins. In contrast, PIC1 overexpressing lines with high Fe in chloroplasts resemble ferritin k.o. plants with low seed yield and imbalanced Fe-homeostasis. Our search for PIC1 interaction partners identified a putative transporter for Ni/Co in the plastid inner envelope. NiCo knockdown mutants show chlorosis and arrest of leaf growth. In comparison to prokaryote-type metal ABC-transporter complexes we further found YGGT, a third membrane-intrinsic component, which we could link to PIC1 and NiCo by immunoprecipitation and purification of native protein complexes. Thus, we propose a chloroplast inner envelope metal transport complex, integrating the function of three proteins most likely involved in Fe transport, metal sensing and membrane-anchoring, respectively.

1.4 TALK 3 – SYMPOSIA 5 – 16:50–17:10

Characterization of the hypothetical manganese exporter MANGA in the cyanobacterium *Synechocystis* sp. PCC 6803

Fabian Brandenburg[1], Marion Eisenhut[1], Ute Krämer[2], Andreas Weber[1]

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[2]Department of Plant Physiology, Bochum, Germany

GreenCutProteins (GCPs) are proteins exclusive to the green lineage. *In silico* analysis of GCPs identified several candidate genes for encoding hypothetical novel transport proteins, which are suggested to play important functions in photosynthetically active organisms. However, most of these proteins consist of domains of unknown functions. We aim at unraveling the biological function of selected GCPs. Using the model cyanobacterium *Synechocystis* sp. PCC 6803, we can demonstrate that the knockout of one of the candidate proteins causes hypersensitivity for manganese (Mn). Accordingly, we named this protein MANGA. In our experiments, the knockout mutant Δ*manga* shows both reduced growth and photosynthetic activity as well as chlorosis following on Mn-treatment. Furthermore, we show that Δ*manga* accumulates high amounts Mn in the cytosol rather than in the periplasm. The phenotype can be repressed by overexpression of MANGA. On the basis of our results we hypothesize that MANGA functions as Mn-exporter in the cytoplasmic membrane of *Synechocystis*. Unable to export Mn into the periplasm, the mutant Δ*manga* accumulates Mn in the cytosol causing Mn-toxicity. To prove Mn-transport activity, MANGA will be heterologously expressed in yeast and transport activity shall be measured.

1.4 TALK 4 – SYMPOSIA 5 – 17:10–17:30

Iron mediates root stem cell differentiation under phosphate starvation

Jens Müller[1], Janine Teller[1], Theresa Toev[1], Katie Moore[1], Steffen Abel[1]

[1]Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

Topsoil foraging is a typical root architectural adaptation to low phosphate (Pi) availability in soil, which comprises attenuation of primary root growth, initiation of lateral roots, and de novo formation of root hairs in *Arabidopsis*. Previous studies revealed that Pi limitation is locally sensed by root tips, causing stem cell differentiation and meristem reduction, a developmental response that also depends on external Fe availability. However, the underlying cellular processes of this interaction are not understood. Our genetic analysis of mutants with contrasting root growth responses to low Pi shows that the P5-type ATPase PHOSPHATE DEFICIENCY RESPONSE 2 (PDR2) and the multicopper oxidase LOW PHOSPHATE ROOT1 (LPR1) functionally interact in a Pi-sensitive pathway to adjust root meristem activity. Recent work demonstrates that the LPR1-PDR2 module specifically regulates apoplastic accumulation/redistribution of Fe in the root stem cell niche under low Pi. We could show that localized accumulation of apoplastic Fe in the root apex likely triggers callose deposition and cell wall modifications in the stem cell niche, which in turn interfere with cell-to-cell communication and induces stem cell differentiation, as revealed by impaired symplastic movement of GFP or a SHR~GFP fusion protein. We propose a mechanism that involves Fe as a mediator of stem cell maintenance in low Pi.

Session 1.5 – Temperature and other Environmental Stresses

Wednesday 10/02 – 10:30-12:00 – Chair: Kerry Franklin

1.5 TALK 1 – SYMPOSIA 6 – 10:30–11:00

Light and temperature-regulation of plant architecture

Kerry Franklin[1]

[1]University of Bristol, Biological Sciences, Bristol, United Kingdom

Light and temperature are two of the most important environmental cues regulating plant development. Our work investigates crosstalk between these signalling pathways. Plants monitor their ambient light environment using specialised information-transducing photoreceptors. A major role of the phytochrome photoreceptors in natural environments is the detection of neighbouring vegetation and initiation of stem elongation responses to avoid shading. Modest changes in ambient growth temperature can also dramatically affect plant architecture. We have shown that high temperature and light quality signalling pathways converge on a shared transcription factor, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) to control elongation growth, via regulation of the plant hormone auxin. The broader interactions of light quality and temperature on hormone signalling and plant development will be discussed.

1.5 TALK 2 – SYMPOSIA 6 – 11:00–11:20

Unraveling molecular mechanisms involved in responses to moderate temperature increases

Carolin Delker[1], Marcel Quint[1]

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In past years climate projections have drawn attention to moderate temperature increases as a potential and so far underestimated abiotic stress. For plants native to areas of moderate climates in particular, mean temperature increases of 2-4°C can have fundamental impact on growth and reproduction. Yet, in contrast to the responses to temperature extremes, molecular mechanisms involved in responses to moderate temperature increases are still poorly understood. A natural variation screen in Arabidopsis, using temperature-induced hypocotyl elongation (TIHE) as a model trait, resulted in the identification of a highly temperature sensitive accession RRS-7. In a subsequent forward genetic approach 37 *okapi* (*opi*) mutants (≥ 6 complementation groups) with compromised TIHE were isolated from EMS-mutagenized RRS-7 seeds. Whole-genome sequencing has resulted in the identification of the first causal mutation. *OPI1* encodes a subunit of an E3 ligase putatively involved in the regulation of expression of several light- and clock-regulated genes implementing a potential integrative cross talk node.

1.5 TALK 3 – SYMPOSIA 6 – 11:20–11:40

Cryptochrome in Arabidopsis thaliana: Is it a sensor for the Geomagnetic Field?

Sunil Kumar Dhiman[1], Paul Galland[1]

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The significant role of cryptochrome as a receptor for the geomagnetic field on the basis of radical pair mechanism has been established. Though cryptochrome is present in plants and performing many important functions, its relationship with geomagnetic fields has not been worked out properly yet. The present work tries to find out what role cryptochrome performs vis a vis light mediated magnetoreception. Stimulus-response curves with respect to magnetic fields for different parameters were generated for the first time. After the exposure to magnetic fields, collected seedlings were measured for their hypocotyl length, tested by RT-PCR for the abundance of specific gene transcripts and two proteins were quantified using western-blotting. The stimulus-response curves for these parameters displayed multiple maxima. The magnetic fields showed highest effects on gene transcription and these affects were found to be reduced in case of hypocotyl length, a feature representing the cumulative result of many genes. So geomagnetic fields affect gene transcription, pigment synthesis and elongation growth in seedlings of *Arabidopsis thaliana*. However our observations don't confirm to the theory of radical pair mechanism based on cryptochrome because the *Arabidopsis* seedlings also respond not only under red light but also in dark. And the cryptochrome 1 and 2 double mutants still reacted to magnetic fields.

1.5 TALK 4 – SYMPOSIA 6 – 11:40–12:00

Multisite phosphorylation of a bZIP transcription factor by different kinases as means for acclimation to different stresses

Andrea Mair[1], Lorenzo Pedrotti[2], Bernhard Wurzinger[1], Dorothea Anrather[1], Andrea Simeunovic[1], Tobias Kirchler[3], Christina Chaban[3], Katrin Dietrich[2], Wolfram Weckwerth[1], Wolfgang Dröge-Laser[2], **Markus Teige**[1]

[1]University of Vienna, Molecular Systems Biology, Vienna, Austria
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[3]ZMBP - University of Tuebingen, Plant Physiology, Plant Physiology, Germany

Question: Protein phosphorylation is a key regulatory principle enabling fast and reversible regulation of a vast number of cellular activities. In response to stress conditions cellular metabolism needs to be switched from normal growth to stress response, for example by the synthesis of protective or defense compounds. We found a bZIP transcription factor to be phosphorylated at multiple sites depending on the energy status of the cell. The question was which kinase(s) phosphorylate this factor and what does this phosphorylation affect? Methods: We applied affinity purification combined with in-gel kinase assays and MS/MS to identify the relevant kinases. Co-localization and interaction was confirmed by different means and direct phosphorylation assays were done to attribute the different sites to the identified kinases. Metabolic profiling and gene expression studies were done to elucidate functional consequences. Results: Of the seven identified in vivo phosphorylation sites, three could be attributed to a SnRK1 kinase and two to a Ca2+-dependent kinase. Only one site targets the central bZIP domain and thereby affects DNA-binding, whereas the other sites affect hetero-dimerization with other bZIP factors. Conclusions: We propose that targeting this bZIP factor by different protein kinases occurs under different stress conditions and enables fast metabolic reprogramming.

2.1 TALK 1 – SYMPOSIA 2 – 16:00–16:30

TAL and other type III effectors from the plant pathogen *Xanthomonas*

Ulla Bonas[1]

[1]University of Halle, Genetics, Halle, Germany

Pathogenicity of most Gram-negative plant pathogenic bacteria depends on a type III secretion system (T3SS), a conserved nanomachine which is induced upon in the plant tissue and translocates effector proteins (T3Es) into the plant cell cytosol. We study the interaction between the model plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and its host plants pepper and tomato. *Xcv* strain 85-10 „injects" more than 25 different T3Es into the plant cell where they manipulate plant cell pathways. In susceptible plants, *Xcv* proliferates and causes disease. In resistant plants, however, resistance genes mediate recognition of a given T3E which often induces a hypersensitive response (HR). The HR is a rapid, localized plant cell death restricting pathogen ingress. T3Es from *Xcv* were identified by their co-regulation with the T3SS (termed Xop, *Xanthomonas* outer protein) or, in case of „Avr" because they induce the HR in resistant plants. AvrBs3 is the founding member of a T3E family in *Xanthomonas*, also termed TAL (transcription activator-like). AvrBs3 activity depends on 17.5 nearly-identical tandem 34-aa repeats in the central region of the protein, nuclear localization signals and an acidic activation domain. We discovered that AvrBs3 acts as a transcription factor in the plant cell nucleus. Recent data on AvrBs3 and interesting Xops (e.g. defense suppressors) will be discussed.

2.1 TALK 2 – SYMPOSIA 2 – 16:30–16:50

Functional characterization of the type III effector protein XopB from *Xanthomonas campestris* pv. *vesicatoria*

Johannes Priller[1], Sabrina Strobl[1], Anja Friedrich[1], Petra Dietrich[1], **Sophia Sonnewald**[1]

[1]FAU Erlangen-Nürnberg, Biologie, Erlangen, Germany

Xanthomonas campestris pv. *vesicatoria (Xcv*) causes bacterial spot disease in tomato and pepper plants. The pathogenicity of *Xcv* depends on a type III secretion system (TTSS) delivering effector proteins (T3E) into plant cells to suppress plant defence and to facilitate bacterial nutrient acquisition. There is increasing evidence that an increased, apoplastic hexose- to-sucrose ratio contributes to plant defense. This ratio is controlled by cell wall-bound invertase (cw-Inv) activity, which was found to be induced in response to pathogen infection. We have recently shown that activity and expression of cw-Inv is increased upon *Xcv* infection of susceptible pepper leaves. However, infection with a TTSS-deficient *Xcv* strain resulted in a higher accumulation of cw-Inv mRNA and enzyme activity compared to Xcv wild type suggesting that bacterial effectors might prevent the generation of sugar-mediated defense responses. In a screen, XopB was identified as strong suppressor of cw-Inv. Thus far the biological and enzymatic activity of XopB is not well understood. Cell-biological and biochemical assays indicate that it is (endo)membrane -associated. In an Y2H screen, an interacting plant protein was identified, which may target XopB to the membrane system. Recent results suggest that XopB may interfere with formation of ROS, Ca2+ signalling and calmalexin biosynthesis.

2.1 Talk 3 – Symposia 2 – 16:50–17:10

Identification of a novel receptor-like kinase that is associated with membrane micro-domains and regulates plant immunity

Corinna Hofer[1], Iris Jarsch[1], Thomas Ott[1]

[1]Ludwig-Maximilians-University Munich, Institute of Genetics, Martinsried, Germany

Emerging evidence indicates the significance of pre-assembled protein complexes within plasma membrane micro-domains to facilitate efficient signal transduction during the interaction between plants and microbes. These domains are targeted by plant-specific remorins which therefore serve as domain marker proteins. Remorins can interact with receptors and other membrane-associated proteins and may serve as molecular scaffolds that mediate complex formation. Here, we used AtREM1.2, a prominent member of this gene family, as a bait in a protein-interaction screen to identify novel signaling proteins that are involved in plant immunity. One LRR-RLK was identified as a specific interaction partner and therefore called Remorin interacting candidate kinase in yeast (RICKY1). RICKY1 belongs to the subgroup LRR-VIII-2 that is characterized by the presence of a malectin domain in its extracellular region. Using imaging based interaction studies we confirmed a specific protein interaction in distinct domains at the plasma membrane. *ricky1* mutant lines are affected in flg22-induced Callose deposition, whereas the early flg22-induced ROS production is not impaired. In line with these data, *ricky1* mutants are significantly more susceptible to *Pseudomonas syringae*. Thus RICKY1 is a novel player in plant immunity.

2.1 TALK 4 – SYMPOSIA 2 – 17:10–17:30

Function of the TGN/EE and an associated RLK FERONIA in plant innate immunity

Nana Keinath[1], Karin Schumacher[1]

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Plants have evolved specialized pattern recognition receptors through which the initial recognition of pathogens via pathogen-associated molecular patterns (PAMPs) takes place. Upon perception the first line of defense is initiated, which is also referred to as PAMP-triggered immunity (PTI). One of the best-characterized PAMP-perception system is the flg22/FLS2 system, of which one hallmark is the receptor endocytosis into endosomes upon elicitor treatment. To date our knowledge about the function of endomembrane compartments in PTI and the dynamics therein is very limited. However, several lines of evidence point to importance of these compartments in pant-pathogen interactions. We have previously established a protocol to affinity purify the TGN/EE with only minimal interference from other organelles[1]. Now, we use organelle-specific proteomics to elucidate the PAMP-induced protein dynamics at the TNG/EE. Preliminary data analysis indicates the accumulation of FERONIA, a receptor-like kinase that we previously identified as a negative regulator of plant innate immunity[2], in the TGN/EE upon flg22 treatment. Detailed mutant analyses and localization studies provide new insights into the role of FERONIA in immune signaling.

1Drakakaki, G. et al. Cell Res 22, 413-424 (2012)

2Keinath, N. et al. J Biol Chem 285, 39140-39149 (2010)

2.2 TALK 1 – SYMPOSIA 3 – 10:30–11:00

How LysM effectors contribute to fungal pathogenicity

Bart Thomma[1], Andrea Sanchez-Vallet[1], Jeroen Mesters[2]

[1]Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands
[2]University of Lübeck, Lübeck, Germany

While multicellular organisms activate immunity upon recognition of pathogen-associated molecular patterns (PAMPs), successful microbial pathogens deliver effector proteins to deregulate PAMP-triggered host immunity and to establish infection. Chitin is the major component of fungal cell walls, and chitin oligosaccharides act as PAMPs in plant cells that are perceived by LysM domain-containing cell surface receptors. We have previously show that the LysM domain-containing effector protein Ecp6 of the fungal plant pathogen *Cladosporium fulvum* mediates virulence through sequestration of chitin oligosaccharides that would otherwise activate chitin-triggered immunity. However, the mechanism by which Ecp6 can compete for chitin binding with host immune receptors remained unclear. Based on structural analysis of Ecp6 we reveal a novel mechanism for chitin binding by intrachain LysM dimerization, leading to a chitin binding groove that is deeply buried in the Ecp6 effector protein. Isothermal titration calorimetry experiments reveal that the ligand-induced composite binding site mediates chitin binding with ultra-high (pM) affinity. Intriguingly, a third, singular, LysM domain of Ecp6 binds chitin with low micromolar affinity but can nevertheless still perturb chitin-triggered immunity. Conceivably, the perturbation by LysM2 is not established through chitin sequestration.

2.2 TALK 2 – SYMPOSIA 3 – 11:00–11:20

The *Arabidopsis thaliana* Receptor-like Protein RLP30 Mediates Innate Immunity towards Necrotrophic Fungi

Weiguo Zhang[1], Malou Fraiture[1], Dagmar Kolb[1], Birgit Löffelhardt[1], Yoshitake Desaki[1], Freddy Boutrot[2], Cyril Zipfel[2], Frederic Brunner[1], **Andrea Gust**[1]

[1]University Tübingen, ZMBP, Plant Biochemistry, Tübingen, Germany
[2]The Sainsbury Laboratory, Norwich, United Kingdom

Effective plant defense strategies rely on the perception of non-self determinants, so-called microbe-associated molecular patterns (MAMPs), by transmembrane pattern recognition receptors (PRRs) and on the resulting induction of corresponding immune responses. Plant resistance against necrotrophic pathogens with a broad host-range is complex and yet not well understood. Here, we have isolated a novel proteinaceous elicitor called SsE1 from the necrotrophic fungal pathogen *Sclerotinia sclerotiorum* that induces typical MAMP-induced immune responses in the model plant *Arabidopsis thaliana*. Analysis of natural genetic variation between different Arabidopsis accessions revealed five ecotypes (Mt-0, Lov-1, Lov-5, Br-0 and Sq-1) that are fully insensitive to SsE1. We used a F2 segregating population from crosses between Col-0 and the SsE1-insensitive ecotype Lov-1 and mapped the locus determining SsE1 sensitivity to RECEPTOR-LIKE PROTEIN 30 (RLP30). We also show that SsE1-triggered immune responses depend on the regulatory receptor-like kinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE 1 (BAK1). Knock-out mutant lines in both *RLP30* and *BAK1* are more susceptible to *S. sclerotiorum* and the taxonomically-related fungus *Botrytis cinerea*. The identification of SsE1 and RLP30 as a potential MAMP-PRR pair demonstrates the relevance of plant MAMP-triggered immunity in the resistance to necrotrophic fungi.

2.2 TALK 3 – SYMPOSIA 3 – 11:20–11:40

Pipecolic acid is an essential regulator of systemic acquired resistance and defense priming in plants

Anne-Christin Doering[1], Friederike Bernsdorff[1], Hana Návarová[1], Jürgen Zeier[1]

[1]Heinrich-Heine University, Molecular ecophysiology of plants, Duesseldorf, Germany

Systemic acquired resistance (SAR) is an inducible immune response that confers broad-spectrum disease resistance against biotrophic and hemibiotrophic pathogens at the whole plant level. The non-protein amino acid pipecolic acid (Pip) possesses two central roles for SAR in *Arabidopsis thaliana*. First, it guarantees SAR establishment via a feedback amplification mechanism, and secondly, it acts as an endogenous mediator of SAR-associated defense priming, enabling the plant to react faster and more strongly to further pathogen attack (Návarová *et al.* 2012). The presented results will highlight how Pip and SA interact to orchestrate SAR and defence priming. Our group has also identified an important role for Pip in basal resistance to the hemibiotrophic bacterial pathogen *Pseudomonas syringae* (Návarová *et al.* 2012). We are now investigating whether Pip is involved in resistance induction to other pathogen types, such as the necrotrophic fungus *Botrytis cinerea* or the oomycete *Hyaloperonospora arabidopsidis*.

Literature: Návarová H., Bernsdorff F., Döring A.-C. & Zeier J. (2012). *The Plant Cell* **24**, 5123-5141.

2.2 TALK 4 – SYMPOSIA 3 – 11:40–12:00

Structure and biochemistry of pathogen-induced callose

Marcel Naumann[1], Natalie Dalüge[1], Dennis Eggert[2], Rudolph Reimer[2], **Christian Voigt**[1]

[1]University of Hamburg, Phytopathology and Biochemistry, Hamburg, Germany
[2]Heinrich Pette Institute, Microscopy and Microtechnology, Hamburg, Germany

In plants, callose, a (1,3)-β-glucan, is deposited in response to abiotic and biotic stress. We could recently proof that callose can have a decisive role in preventing fungal penetration. The overexpression of the callose synthase GSL5, responsible for stress-induced callose formation in *Arabidopsis thaliana,* resulted in an elevated early callose deposition at sites of attempted powdery mildew infection and complete penetration resistance. Here, we wanted to know whether GSL5 overexpression would also affect the structure of callose deposits. The non-invasive method of direct stochastic optical reconstruction microscopy (dSTORM) facilitated a resolution of < 50 nm for aniline blue-stained callose polymer fibres. Only callose deposits of the resistant GSL5 overexpression lines revealed distinct structural differences in the orientation of callose fibres between the central core of the callose deposit and surrounding field of callose. Biochemical characterization of GSL5 further revealed that the hydrophilic loop of the callose synthase GSL5 is sufficient for callose biosynthesis. Interestingly, the cellulose precursor cellubiose, a (1,4)-β-glucose dimer, facilitated a higher callose synthase activity than the callose precursor laminaribiose, a (1,3)-β-glucose dimer. This indicates a putative interaction of callose formation with cellulose biosynthesis.

2.3 TALK 1 – SYMPOSIA 5 – 16:00–16:30

Using Plant-mediated RNAi in the field to study plant-insect interactions

Ian Thomas Baldwin[1], Pavan Kumar[1], Sagar Pandit[1]

[1]Max-Planck-Institute for Chemical Ecology, Department of Molecular Ecology, Jena, Germany

Plant-mediated RNAi provides a convenient solution to the festering problem that thwarts functional genomic studies of Lepidoptern herbivorous insects: their lack of RdRs or other molecular machinery to amplify injected or ingested smRNAs that would allow for sustained gene silencing. By transforming host plants to express insect dsRNAs at high levels, we created a convenient means of silencing insect genes that are specifically up-regulated during plant-insect interactions. The talk will present an example of this approach used in the field, and how field work proved to be essential in understanding the organismic-level function of the up-regulated gene.

2.3 TALK 2 – SYMPOSIA 5 – 16:30–16:50

Regulation of nectar composition of day- and nightflowering plants

Kira Tiedge[1], Horst Fuhrmann[1], Gertrud Lohaus[1]

[1]Bergische Universität Wuppertal, Plant Biochemistry/ Molecular Plant Sciences, Wuppertal, Germany

Nectar is a reward for the plants pollinators thus beside water it may contain sugar (e.g. glucose, fructose, sucrose), cations and anions, amino acids, proteins and secondary metabolites. The aim is to determine the parameters, which are decisive for the nectar composition. Therefore closely related species of day- and nightflowering plants are compared in matters of the mentioned components. Samples are taken from the profound examined genus Nicotiana (part of the family Solanaceae) as well as from the family Onagraceae. The applied methods for nectar analysis are high-performance-liquid-chromatography and protein gel electrophoresis. Nectar of nightflowering plant species mostly is sucrose dominated. This corresponds to the preference of their main pollinators (Sphingidae). Nightflowering, sphingophile (moth pollinated) plants, such as *Oenothera biennis,* turned out to change their nectar composition and concentration in the course of the day. For instance the sugar concentration of this species increases exponentially in the evening hours. The possible reasons for differences between the nectar composition of day- and nightflowering plants will be discussed.

2.3 TALK 3 – SYMPOSIA 5 – 16:50–17:10

Plant metabolic responses to interactions between phytohormone pathways and impacts on herbivores of different feeding types

Rabea Schweiger[1,2], Anna-Maria Heise[1], Marcus Persicke[2], **Caroline Müller**[1,2]

[1]Bielefeld University, Chemical Ecology, Bielefeld, Germany
[2]Bielefeld University, Center of Biotechnology, Bielefeld, Germany

The phytohormones jasmonic acid (JA) and salicylic acid (SA) interact in a complex manner, whereby negative cross-talk between the corresponding signaling pathways has been mainly demonstrated using gene expression studies. Using a metabolomics approach, we compared the responses of *Plantago lanceolata* plants induced either by JA or SA, or by the combination of both to disentangle the interactions between the pathways, which revealed highly specific induction patterns and mutual antagonistic cross-talk effects. Moreover, the consequences of these patterns were tested on survival of herbivores in bioassays. Sucking and chewing herbivores suffered more from single phytohormone applications than from the combined JA + SA treatment. The treatment-specific effects on herbivores may be explained by the fine-tuned induction patterns of metabolites and antagonistic interactions between the JA and SA pathways.

2.3 TALK 4 – SYMPOSIA 5 – 17:10–17:30

New roles for cytokinins in plant responses to wounding and herbivore perception

Martin Schäfer[1], Christoph Brütting[1], Ivan D. Meza-Canales[1], Klaus Gase[1], Radka Vankova[2], Ian T. Baldwin[1], Stefan Meldau[1]

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[2]Institute of Experimental Botany AS CR, Laboratory of hormonal regulations in plants, Prague, Czech Republic

Cytokinins (CKs) are well-known for regulating many developmental and abiotic stress-related processes. Recently they were also found to prime plant immunity against pathogens. In contrast, knowledge about their functions in plant responses to herbivore feeding is mainly elusive. Here we show that in *Nicotiana attenuata*, as well as in *Arabidopsis thaliana* the levels of several active CKs increase after wounding and perception of herbivore elicitor. A more detailed analysis in *N. attenuata* revealed that transcripts of many CK-related genes, including the homolog of the Arabidopsis response regulator 5 are induced by wounding and simulated herbivory. By genetically manipulating CK levels and CK perception in *N. attenuata*, we could show that CKs play essential roles in regulating wound- and herbivory-induced defense responses. We also demonstrate the importance of CKs in controlling defenses during ontogenic stage transitions. Thus our data highlight the role of CKs in regulating plant responses to wounding and herbivore perception.

2.4 TALK 1 – SYMPOSIA 7 – 13:30–14:00

Molecular Genetics of Rhizosphere Communication in Arbuscular Mycorrhizal Symbioses of Cereals

Uta Paszkowski[1]

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The rhizosphere ubiquitously contains a plethora of chemical signals that originate from a wide variety of organisms. Detection and discrimination of species-specific signals is crucial for the activation of the appropriate plant response either to combat pathogens or to attract and facilitate association with beneficial organisms. Likewise, distinguishing host from non-host may be vital for survival, as is the case for obligate endophytes. In beneficial interactions, such as the arbuscular mycorrhizal (AM) symbiosis, mutual pre-symbiotic recognition precedes a well-orchestrated exchange of signals that ascertains the coordination of the interacting organisms for this life-long physical alliance. The nature of some of the signals has been discovered in recent years, providing a first insight into the type of chemical language spoken between the two symbiotic partners. Importantly, these discoveries suggest that the dialogue is complex and that additional factors and corresponding receptors remain to be unveiled. I will introduce two new components from cereals that play a role in "speaking" and "listening" of plants during rhizosphere communication with Glomeromycotan fungi.

2.4 TALK 2 – SYMPOSIA 7 – 14:00–14:20

Disorganized arbuscules is required for arbuscule branching

Caroline Gutjahr[1], Andreas Keymer[1], Aline Banhara[1], Vera Wewer[2], Simone Hardel[1], Edda von Roepenack-Lahaye[3], Peter Dörmann[2], Martin Parniske[1]

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[2]University of Bonn, Institute of Molecular Physiology and Biotechnology of Plants, Bonn, Germany
[3]University of Munich (LMU), Botany, Munich, Germany

Arbuscular mycorrhiza development culminates in the formation of highly branched fungal structures, the arbuscules in cortical cells. Previous forward and reverse genetics approaches using plant mutants, unraveled distinct phases of arbuscule development that are under plant control and can be genetically dissected into PPA formation, fungal cell entry, arbuscule branching and collapse. In a forward genetics screen we identified a novel *Lotus japonicus* mutant called *disorganized arbuscules* (*dis*) that is impaired in arbuscule branching. We mapped and used next generation sequencing to identify the causative mutation. The *DIS* gene, encodes an enzyme involved in fatty acid biosynthesis, of which three paralogues are present in the *Lotus japonicus* genome. Promoter-*GUS* localization studies showed that the *DIS* promoter is active in arbuscule containing cells. A DIS-GFP-fusion was targeted to plastids, consistent with the subcellular localization of fatty acid biosynthesis. Progress towards unraveling the role of the DIS protein in arbuscular mycorrhiza will be presented.

2.4 TALK 3 – SYMPOSIA 7 – 14:20–14:40

Sugar for my honey: Mechanisms of fungal carbohydrate nutrition in ectomycorrhizal symbiosis

Uwe Nehls[1], Sebastian Nintemann[1], Jennifer Krützmann[1], Christian Zenker[1], Annette Hintelmann[1]

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Ectomycorrhizal symbiosis is a mutualistic interaction between soil fungi and fine roots of forest trees. Essential for the symbiosis is a sustainable carbohydrate support of the fungal partner, which consumes up to 15 % of host plant assimilates. While sugars are expected to be the predominant fungal carbohydrate source in symbiosis, the nature and the pathway by which this source is delivered by the plant partner are still unknown. Whole genome expression analysis using Nimble Gene Arrays revealed that genes encoding proteins involved in both sucrose and starch metabolism were up-regulated upon mycorrhiza formation in poplar. Sucrose break down, which is mainly hydrolytic in non-mycorrhized fine roots turned out to become phosphorolytic in mycorrhizas. Furthermore, expression of certain members of a gene family coding for putative sugar efflux carriers was highly induced upon ectomycorrhiza formation. Mycorrhiza-specific gene expression was confirmed by quantitative RT-PCR. The respective proteins were localized in the plant plasma membrane after transformation of tobacco leaves with translational YFP fusions. Functional analysis of the proteins by heterologous expression of the corresponding reading frames in yeast revealed their function as glucose facilitators. Hence, we propose glucose as plant-derived carbohydrate released upon ectomycorrhizal symbiosis and members of the hexose facilitator gene family as respective carbohydrate exporters.

2.4 TALK 4 – SYMPOSIA 7 – 14:40–15:00

General and plant species-specific foliar metabolic responses to arbuscular mycorrhiza

Rabea Schweiger[1], Markus C. Baier[1], Caroline Müller[1]

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Arbuscular mycorrhizal (AM) fungi are commonly associated with higher land plants and regarded to be integrative parts of the plant's physiology. Mycorrhizal effects on plant chemistry have been mainly analyzed at the root level, while little is known about how the shoot metabolome responds to the symbiotic association. The improved nutrient supply and fungal carbon sink activity may affect resource allocation and thus influence plant primary and secondary metabolites at the whole plant level. Hence, in a species-comparison approach, we assessed foliar metabolic changes of five herbaceous plant species with different phylogenetic relationships using comparative metabolomics to evaluate the specificity of plant responses to AM fungi. *Rhizophagus intraradices* intensely colonized the roots of all plant species at comparable levels. AM increased shoot phosphorus levels in all plant species, whereas carbon and nitrogen contents were not affected. Using metabolite profiling and metabolic fingerprinting, we found sets of metabolites changed along with mycorrhization commonly in all plant species, which can be considered as mycorrhizal plant biomarkers, as well as metabolites specifically in- or decreased only in certain plant species. These metabolic changes should have important consequences for the ecological outcome of plant-herbivore and plant-pathogen interactions.

2.5 TALK 1 – SYMPOSIA 8 – 15:30–16:00

Bacterial infection of epidermal cells is controlled by a two-step recognition process

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Development of root nodules in legumes in response to signals secreted from rhizobia is an example of a regulated bacterial infection process that is synchronised with an inducible organ formation. Lipochito-oligosaccharides (Nod-factors), consisting of substituted b,1-4 N-acetylglucosamine (chitin) backbones are the major rhizobial signals triggering root hair deformation and initiation of the bacterial infection process. An important determinant of bacterial recognition and host specificity is the interaction between Nod-factors and plant receptors involved in signal perception and signal transduction. The role of the two *Lotus japonicus* LysM type serine/threonine receptor kinases, NFR1 and NFR5, in perception of Nod-factor signals from bacterial microsymbionts will be discussed. The extracellular domains of the two trans-membrane kinases carries LysM domains suggesting that they are involved in binding of the rhizobial lipochitin-oligosaccharide signals. Biochemical experiments addressing this question will be presented and the involvement of NFR1 and NFR5 receptor kinases in the earliest physiological and cellular responses will be illustrated. A second recognition step has now been uncovered. The molecular basis of this non-self recognition will be presented together with a model for a two-step recognition of rhizobial bacteria at initiation of infection.

2.5 TALK 2 – SYMPOSIA 8 – 16:00–16:20

Activation of CYCLOPS, a novel DNA-binding transcriptional regulator leads to spontaneous nodule formation in the absence of rhizobia

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Nuclear calcium oscillations are a hallmark of symbiotically stimulated plant root cells. Activation of the central nuclear decoder, calcium- and calmodulin-dependent kinase (CCaMK), triggers the entire symbiotic program including root nodule organogenesis, but the mechanism of signal transduction by CCaMK was unknown. We show that CYCLOPS, a phosphorylation substrate of CCaMK, constitutes a novel class of DNA-binding transcriptional activator. Two phosphorylated residues within the N-terminal negative regulatory domain are necessary to release CYCLOPS from autoinhibition. A phospho-mimetic version was solosufficient for triggering root nodule organogenesis in the absence of rhizobia and CCaMK. Our data pinpoint the CCaMK/CYCLOPS complex as central regulatory node which directly translates nuclear calcium oscillations into the activation of the *NODULE INCEPTION* (*NIN*) gene. CYCLOPS thus emerges as the master regulator of a cascade of transcriptional regulation, in which NIN and a heterotrimeric NF-Y complex act in hierarchical succession to initiate symbiotic root nodule development.

2.5 TALK 3 – SYMPOSIA 8 – 16:20–16:40

Regulation of Agrobacterial *Ipt* Oncogene Expression in Host plants

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In nature several economically important plant species such as walnut or grapevine become transformed with T-DNA by virulent *A. tumefaciens* strains and suffer from the crown gall disease. Genes responsible for this tumor disease are the three T-DNA-encoded oncogenes, *iaaM*, *iaaH*, and *Ipt*. The aim of our studies is to unravel the molecular mechanism regulating expression of oncogenes in host cells. Our studies demonstrate that the *Ipt* promoter has a eukaryotic sequence structure comprising AuxREs and W-boxes for binding of plant transcription factors. We identified two candidates from the WRKY and ARF transcription factor family which show elevated gene expression in tumors and activate the *Ipt* promoter. Tumors of *wrky* mutant plants are smaller than those of the wild type, suggesting a role of WRKY in tumor development. WRKY protein binds to the *Ipt* promoter *in vitro* and interacts with ARF in the plant cell nucleus. In the presence of auxin both transcription factors synergistically activate the *Ipt* promoter. The activation by ARF alone is inhibited by Aux/IAA. In our model we suggest that elevated auxin levels in T-DNA transformed *Arabidopsis* cells induce degradation of Aux/IAA. Aux/IAA degradation releases ARF to form a complex with WRKY. The WRKY/ARF complex binds to the W-boxes and AuxREs in the *Ipt* promoter to induce expression of the oncogene in plant host cells.

2.5 TALK 4 – SYMPOSIA 8 – 16:40–17:00

The *Xanthomonas campestris* Type III Effector XopJ Targets the Host Cell Proteasome to Suppress Salicylic-Acid Mediated Plant Defence

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The phytopathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (Xcv) requires type III effector proteins (T3Es) for virulence. After translocation into the host cell T3Es are thought to interact with components of host immunity to suppress defence responses. XopJ is a T3E protein from Xcv that interferes with plant immune responses; however, its host cellular target is unknown. Here we show that XopJ interacts with the proteasomal subunit RPT6 in yeast and *in planta* to inhibit proteasome activity, dependent on the catalytic activity and localisation of XopJ. Xcv Δ*xopJ* mutants are impaired in growth and display accelerated symptom development including tissue necrosis on susceptible pepper leaves. Application of the proteasome inhibitor MG132 restored the ability of the Xcv Δ*xopJ* to attenuate the development of leaf necrosis. The XopJ dependent delay of tissue degeneration correlates with reduced levels of salicylic acid (SA) and changes in SA- and senescence-associated gene expression. Necrosis upon infection with Xcv Δ*xopJ* was greatly reduced in pepper plants silenced for *NPR1*, a central regulator of SA responses, demonstrating the involvement of SA-signalling in the development of XopJ-dependent phenotypes. Our results suggest that XopJ-mediated inhibition of the proteasome interferes with SA-dependent defence responses to attenuate the onset of necrosis.

2.6 TALK 1 – SYMPOSIA 6 – 10:30–11:00

Endophytic fungi are involved in multiple balanced antagonisms

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In order to infect, grow and survive within their plant hosts, fungal endophytes must deal with the plant's physical barriers and defense responses. Whereas endophytic colonization of the host often activates host defense reactions, endophytic fungi interact by secreting toxic metabolites, exoenzymes and phytohormones as virulence factors. However, in studying the interactions, it is important to consider the *in situ* situation. For example, fungal endophytes must also deal with bacterial and fungal competitors and also secrete metabolites active such microorganisms, which in turn secrete metabolites toxic to the endophytes. Environmental factors may also influence the outcome of these delicate balances of antagonism, and thus whether disease develops or the plant remains healthy. Thus, we hypothesize that in order to grow asymptomatically within the host plant, endophytic fungi are involved in multiple balanced antagonisms, responding with phenotypic plasticity to the respective situation. Pathogens are specialists; endophytes are masters of adaption.

2.6 TALK 2 – SYMPOSIA 6 – 11:00–11:20

The endophyte *Acremonium alternatum* affects plant growth and pathogen infection with clubroot

Susann Auer[1], Jutta Ludwig-Müller[1]

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The clubroot pathogen *Plasmodiophora brassicae* infects economically important Brassica crops such as canola and causes high yield losses. Infected plants show abnormal root growth whereas upper plant parts wither. The disease is difficult to control by chemical and cultural means and continues to spread around the globe. Infested fields can effectively no longer be used for cultivation of energy and oil plant canola for the next five years or more. Despite costly breeding of resistant cultivars, recent research leans towards alternative, low-impact, environmentally friendly methods to control clubroot. In a previous study *Acremonium alternatum*, an endophyte and known biological control agent in other countries, showed a promising antagonistic effect in clubroot infected Arabidopsis and Chinese cabbage. The means by which *Acremonium* controls pathogens is not known so far. In clubroot infected plants the fungus delays the development of *Plasmodiophora,* presumably by inducing resistance mechanisms of the host. We found several resistance genes to be differentially expressed in the tripartite interaction of *Plasmodiphora-Acremonium*-Arabidopsis. In addition the fungus seems to increase the abiotic stress tolerance of plants. The long-term goal is to contribute to an effective reduction of clubroot to be used in integrated pest management.

2.6 TALK 3 – SYMPOSIA 6 – 11:20–11:40

Latent bacterial background of "axenic" plant cell cultures: Contaminants or Endophytes?

Nicole Brinkmann[1], Maja Marheine[1], Elke Heine-Dobbernack[1], Heinz Martin Schumacher[1]

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Since more than 20 years about 700 undifferentiated plant cell lines are maintained routinely in the DSMZ plant cell culture collection. Contaminated cell cultures are discarded immediately before contaminations lead to culture losses. However, in contrast to sporadically occurring contaminants, caused by laboratory failure, some out-sorted plant cell lines seemed to carry latent bacterial infections of mutualistic nature. Application of molecular approaches and improved culturing reveals the hidden presence of an unexpected bacterial diversity in calli of various plant families. Bacterial diversity in calli includes species of *Mycobacterium* and *Pseudomonas*, as well as a methanogenic bacterium and even new species of *Paenibacilli*. Persisting microorganisms may be endophytic bacteria staying invisible without symptoms of callus damage for more than 20 years of subcultivation; however, their function is still unclear. SEM analyses showed different colonization densities of bacteria depending on plant callus. An exact quantification of single species by q-PCR using specific probes is in progress. Currently, microhabitats of varying pH, O2 and CO2 within calli were identified that shall be correlated with the spatial distribution of endophytic bacteria in thin sections of calli using FISH.

2.6 TALK 4 – SYMPOSIA 6 – 11:40–12:00

Host specificity of phyllosphere fungal communities of tropical trees

Dominik Begerow[1], Tesfaye Wubet[2]

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Tropical microorganisms in the phyllosphere such as epiphyllous yeast and endophytic filamentous fungi contribute substantially to global organismic diversity. Because of their presence in virtually every ecosystem and the production of enzymes, alkaloids, antibiotics, phytohormones and other secondary metabolites, they markedly increase functional diversity of the inhabited vegetation. However, an accurate estimate of phyllosphere fungi of tropical trees is lacking and their ecology is almost unknown. Using next generation sequencing technology we analysed two tree species of a tropical mountain rainforest in southern Ecuador to estimate the amount of fungal species per host plant species and to analyse the fungal phyllosphere communities in terms of host specificity and impact of microclimate on the fungal community composition. Based on leaf samples sampled from12 trees, we could identify more than 1000 fungal OTUs of asco- and basidiomycetes. Each tree species was characterized by statistically significant distinctive fungal community. The significance of host tree species, microclimate condition and neighbouring tree effect on the fungal community will be discussed.

3.1 TALK 1 – SYMPOSIA 3 – 10:30–11:00

Trichome Patterning in Arabidopsis: how protein-protein interactions shape the dynamics of a network

Martin Hülskamp[1], Martina Pesch[1], Ilka Schultheiss[1], Divykriti Chopra[1], Andrea Schrader[1]

[1]University of Cologne, Botanical Institute, Cologne, Germany

We use trichome patterning in *Arabidopsis thaliana* as a model system to understand how a gene regulatory network governs the establishment of a two dimensional pattern. It is postulated that positive and negative regulators are engaged in feed back loops with small R3MYB proteins mediating the intercellular communication. We focus on the question, how protein-protein interactions between the core components and with other proteins shape the molecular dynamics of the network.

3.1 TALK 2 – SYMPOSIA 3 – 11:00–11:20

Temporal control of leaf complexity by miRNA-controlled licensing of protein complexes

Ignacio Rubio Somoza[1], Chuan-Miao Zhou[2], Ana Confraria[3], Claudia Martinho[3], Patrick von Born[1], Elena Baena-Gonzalez[3], Jia-Wei Wang[1,2], Detlef Weigel[1]

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[3]Instituto Gulbenkian de Ciência, Oeiras, Portugal

Post-embryonic development in plants is characterized by the production of new organs such as leaves from the pool of stem cells embedded in the shoot apical meristem. The transition from juvenile to adult growth is accompained by the age-dependent acquisition of diverse features in the newly formed organs. In *Arabidopsis thaliana*, leaf margins become progressively serrated, and specialized cell types such as abaxial trichomes appear. In the *A. thaliana* relative *Cardamine hirsuta*, marginal lobes develop instead of mere serrations. We have investigated how an evolutionarily conserved group of microRNAs (miRNAs) orchestrates the gradual gain of leaf complexity in both plant species during heteroblasty. Two miRNA nodes modulate complexity, with temporal control exerted by another miRNA-transcription factor node. Our results point to miRNA networks as being at the center of genetic programs that control developmental timing. In addition, our results reveal how miRNA-transcription factor nodes interact to form regulatory networks.

3.1 TALK 3 – SYMPOSIA 3 – 11:20–11:40

Palmitoylation of Remorin proteins stabilizes plasma membrane binding but does not confer localization to membrane domains.

Sebastian Konrad[1], Claudia Popp[1], Thomas F. Stratil[1], Thomas Ott[1]

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In every cell, signal transduction relies on the formation of higher order protein complexes. The preformation of signaling complexes in so-called membrane domains is thought to accelerate signal transduction. Remorin proteins have been shown to be components of key signaling complexes and are widely used as membrane domain marker, that allow to study the compartmentalization of the plasma membrane *in vivo*. Many membrane domain localized proteins are reported to bind membranes via posttranslational lipid modifications. We show, that S-Acylation (commonly referred to as palmitoylation) of C-terminal residues is a general feature of Remorin proteins and crucial for their final attachment to the plasma membrane. Although it has been widely suggested that palmitoylation may determine membrane-domain localization of proteins, we show that S-acylation of Remorins serves as an auxiliary modification to stabilize membrane association, but is not required for their localization in membrane domains. Our data suggest that this feature is mediated by protein-protein interactions.

3.1 TALK 4 – SYMPOSIA 3 – 11:40–12:00

Genomics and transcriptomics to analyze multicellular development in fungi

Minou Nowrousian[1], Stefanie Traeger[1], Florian Altegoer[1], Ines Teichert[1], Gabriele Wolff[1], Ulrich Kück[1]

[1]Ruhr-Universität Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany

During sexual development, filamentous ascomycetes form complex, three-dimensional fruiting bodies for the protection and dispersal of spores. We are using next generation sequencing to study this differentiation process in the ascomycetes *Sordaria macrospora* and *Pyronema confluens*. Whole genome sequencing of the *S. macrospora* mutant pro44 was used to identify the mutation causing sterility in the mutant through bulk segregant sequencing. pro44 carries a mutation in a transcription factor, and fertility can be restored by transformation with the wild-type allele. In a second approach, we used laser microdissection to isolate young fruiting bodies (protoperithecia) of the wild type and mutant pro1 carrying a deletion of another developmental transcription factor gene. Analysis of RNA-seq reads from microdissected samples showed that *pro44* is among the 500 most strongly expressed genes in wild-type, but not pro1. *De novo* genome sequencing of *P. confluens* and RNA-seq of different developmental stages showed that the *P. confluens pro44* ortholog is also upregulated during sexual development. The *P. confluens pro44* complements the *S. macrospora* pro44 mutant indicating a conserved function in development. In summary, our data indicate that PRO1 and PRO44 are members of a transcription factor network that regulates gene expression and differentiation in developing fruiting bodies.

3.2 TALK 1 – SYMPOSIA 3 – 10:30–11:00

MicroRNA regulation of leaf development

Patrick Laufs[1], Bernard Adroher[1], Nicolas Arnaud[1], Thomas Blein[1], Millan Cortizo[1], Alice Hasson[1], Marie-Capucine Lepeigneux[1]

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Since their identification one decade ago, microRNAs have emerged as important regulators of plant development. By acting both locally and at the whole organ scale, they have an essential role in the coordination of complex developmental processes and are integrated in genetic networks and signalling pathways. Here, the complex functions of miRNAs will be exemplified by taking leaf development as a model system. First, a general overview of leaf development will be given, highlighting different contributions of miRNAs. Second, we will focus on a class of miRNA, *miR164* that regulates several genes coding for NAC transcription factors. Among these miR164 targets are the *CUC1* and *CUC2* genes that together with the non-*miR164* target *CUC3* define the boundary domain separating the leaf primordium from the meristem and act later to serrate the leaf margin. The role and the interactions between *CUC* genes and *miR164* during leaf development will be described

3.2 TALK 2 – SYMPOSIA 3 – 11:00–11:20

Regulative key factors of leaf senescence which are putative targets for chromatin alterations in Arabidopsis

Nicole Ay[1], Ulrike Raum[1], Andreas Fischer[1], Salma Balazadeh[2], Gunter Reuter[1], Klaus Humbeck[1]

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Leaf senescence involves extensive reprogramming of gene expression effectuating the complex biochemical and structural changes occurring during the last step of leaf development. In a large-scale transcriptomic approach in Arabidopsis, using qRT-PCR, more than 300 transcriptional regulators were shown to be regulated in a senescence-specific manner. Overexpression of the histone methyltransferase SUVH2, which was previously reported to delay leaf senescence (Ay et al. 2009), impaired the expression of about 50% of these senescence related regulatory factors (SRRFs). Thereby, the senescence-associated transcription factor families AP2-EREBP, C2H2, NAC and WRKY are affected most notably. This suggests an either direct or indirect, locus-specific mode of SUVH2 action. Interestingly, 45 genes of the identified SRRFs possess an ERF-associated amphiphilic repression (EAR) motif, indicating that EAR motif-mediated transcriptional repression could be a principal mechanism within regulation of senescence. Furthermore, about 30% of the SRRFs are predicted as putative targets of the ELONGATED HYPOCOTYL5 (HY5) bZIP transcription factor, which implies an important role of HY5 within the regulatory senescence network. Our data reinforce the involvement of epigenetic processes during regulation of senescence-specific gene expression in Arabidopsis and give new insights into the regulatory network of leaf senescence.

3.2 TALK 3 – SYMPOSIA 3 – 11:20–11:40

Approach to precision-engineering of plant minichromosomes

Michael Florian Mette[1], Chee How Teo[1], Eszter Kapusi[1], Lu Ma[1], Götz Hensel[1], Axel Himmelbach[1], Jochen Kumlehn[1], Ingo Schubert[1], Andreas Houben[1], Inna Lermontova[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Stadt Seeland OT Gatersleben, Germany

Custom-designed minichromosomes are highly desirable tools for the analysis of plant chromosome function and for plant biotechnology [1]. We have established protocols to generate functional *de novo* centromeres *via* targeting of recombinant kinetochore proteins to tandem repeat arrays at non-centromeric positions [2] as well as stably transmissible random-truncated chromosomes *via* T-DNA-mediated telomere seeding [3;4]. Now, we aim to combine these methods with TALEN-mediated targeting of chromosome truncation to engineer minichromosomes with high precision in the model *Arabidopsis thaliana* and in barley as a crop. Further, the requisites for efficient *de novo* centromere formation will be analysed and mitotic and meiotic activity of *de novo* centromeres be determined.

[1] Houben et al. (2013) Engineered plant minichromosomes. *Int. J. Dev. Biol.* in press

[2] Teo et al. (2013) *De novo* generation of plant centromeres at tandem repeats. *Chromosoma* 122:233-241

[3] Kapusi et al. (2012) Telomere-mediated truncation of barley chromosomes. *Chromosoma* 121:181-190

[4] Teo et. al (2011) Induction of telomere-mediated chromosomal truncation and stability of truncated chromosomes in *Arabidopsis thaliana*. *Plant J.* 68:28-39

3.2 TALK 4 – SYMPOSIA 3 – 11:40–12:00

Short non-coding RNA fragments accumulating in plant organelles: footprints of RNA binding proteins

Hannes Ruwe[1], Sandra Gusewski[1], Yujiao Qu[1], **Christian Schmitz-Linneweber**[1]

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Question: Chloroplast RNA metabolism is controlled and executed by hundreds of nuclear-encoded, chloroplast-localized RNA binding proteins. Contrary to the nucleo-cytosolic compartment, there is little evidence for noncoding RNAs, e.g. miRNAs-likes, to play a role as riboregulators in chloroplasts. Methods and Results: We mined deep-sequencing datasets to identify short (16-28 nt) RNAs (sRNAs) in the chloroplast genome and found more than 100 small chloroplast RNAs, some of them extremely abundant. Most of these sRNA are located in non-coding regions and many are found upstream of start codons. By transcript end mapping we show that 5' and 3' termini of chloroplast RNAs coincide with ends of sRNAs. Sequences of sRNAs identified in Arabidopsis are conserved between different angiosperm species and in several cases, we identified putative orthologs in rice deep sequencing datasets. Conclusions: Recently, it was suggested that small chloroplast RNA fragments could result from the protective action of pentatricopeptide repeat (PPR) proteins against exonucleases, i.e. be footprints of RNA binding proteins (Pfalz et al. EMBO J 28:2042-52; 2009). Our data support this scenario on a transcriptome-wide level and suggest that a large number of sRNAs are in fact remnants of mRNAs targeted by PPR proteins. Similar findings are presented for plant mitochondria.

3.3 TALK 1 – SYMPOSIA 5 – 16:00–16:30

Vacuoles - Pumping up the plant volume

Karin Schumacher[1]

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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 recent insights into the process of vacuole biogenesis.

3.3 TALK 2 – SYMPOSIA 5 – 16:30–16:50

Phosphoinositide control of PIN polarization in Arabidopsis

Till Ischebeck[1], Stephanie Werner[2,3], Praveen Krishnamoorthy[4], Staffan Persson[4], **Ingo Heilmann**[2]

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[2]Martin-Luther-University Halle-Wittenberg, Biochemistry, Halle (Saale), Germany
[3]Gregor-Mendel-Institute for Molecular Plant Biology, Vienna, Germany
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The minor phospholipid, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2), controls directional membrane trafficking in polar growing cells. However, functions of PtdIns(4,5)P2 during vegetative growth of plants are less well characterized. PtdIns(4,5)P2 contributes to the distribution of auxin efflux carriers (PIN-formed protein or PINs), suggesting that by influencing auxin homeostasis phosphoinositides have profound influence on vegetative plant growth and development. Based on lipid analysis, genetic evidence and in vivo imaging, we are trying to understand how phosphoinositides and auxin signaling interact. The presentation will provide an overview of the latest findings on how PtdIns(4,5)P2 contributes to the polarization of PIN proteins.

3.3 TALK 3 – SYMPOSIA 5 – 16:50–17:10

Regulation of endocytic sorting and trafficking of the immune receptor FLAGELLIN SENSING 2

Martina Beck[1], Thomas Spallek[2], Jenna Loiseau[1], Silke Robatzek[1]

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The immune receptor FLAGELLIN SENSING 2 (FLS2) plays important roles in plant resistance to bacterial pathogens and is internalized from the plasma membrane via the endocytic pathway when triggered by its ligand flg22 (Robatzek et al., 2006). Recently, we defined the FLS2 endocytic pathway showing that FLS2 traffics via two distinct endocytic routes depending on its activation status (Beck et al., 2012). FLS2 receptors constitutively recycle in a Brefeldin A (BFA)-sensitive manner, while flg22-activated receptors traffic via ARA7/RabF2b- and ARA6/RabF1-positive endosomes. Endocytosis of FLS2 requires a functional ARA7/RabF2b. The central hub for sorting endosomal cargo is represented by the *trans*-Golgi Network/early endosomes (TGN/EE). Here, we show that both endocytic routes of FLS2 share the TGN/EE, but non-activated FLS2 is recycled back to the plasma membrane, whereas the activated receptors are transported and sorted to the lumen of multivesicular bodies/late endosomes (MVB/LE). MVB sorting of FLS2 is mediated by the ENDOSOMAL SORTING COMPLEX REQUIRED FOR TRANSPORT (ESCRT)-I component VPS37-1. We will discuss these recent results and aspects, by which ESCRT-I regulates FLS2 endocytosis and plant immunity.

Robatzek et al (2006) *GenDev* 20(5):537-542; Beck et al. (2012) Plant Cell;24(10):4205-19

3.3 TALK 4 – SYMPOSIA 5 – 17:10–17:30

A new class of microtubule associated proteins regulates the interaction between the plasma membrane and cortical microtubules in Arabidopsis

Zengyu Liu[1], Staffan Persson[1]

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The microtubule cytoskeleton is essential for directed cell growth that sustains cell and organ shapes. In interphase cells the main bulk of microtubules is found at the cell cortex where they assist cellulose synthesis that occurs via cellulose synthases at the plasma membrane. As the CESAs are physically linked to the microtubules via a linker protein, they could also work as scaffolds for maintaining the microtubules at the cortex. However, disruption of this link still maintains cortical microtubule arrays and other types of connections between the plasma membrane and the cortical microtubules are therefore anticipated. Here, we identified a *de novo* microtubule associating protein 1 (*nMAP1*) and its homologue, de novo microtubule associating protein 2 (*nMAP2*), which potentially mediate this important interaction. Both GFP-nMAP1 and GFP-nMAP2 co-localize with microtubules in vivo. This association was confirmed by co-immunoprecipitation using GFP-nMAP1 as bait. Mutations in *nMAP1* and *nMAP2* led to plants with microtubule defects when grown on oryzalin containing MS plates. Interestingly, the cortical microtubules in the double mutants displayed extreme bending and frequently showed ends that pivoted away from straight polymerization behavior. This abnormal phenotype might indicate that the association between microtubules and plasma membrane is disturbed by the mutations in *nMAP1* and *nMAP2*.

3.4 TALK 1 – SYMPOSIA 7 – 13:30–14:00

Interactions of the plant cytoskeleton with membranes

Patrick J. Hussey[1]

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Complex animals use a wide variety of adaptor proteins to produce specialized sites of interaction between actin and membranes. Plants do not have these protein families, yet actin-membrane interactions within plant cells are critical for the positioning of subcellular compartments, for coordinating intercellular communication, and for membrane deformation. Novel factors are therefore likely to provide interfaces at actin-membrane contacts in plants, but their identity has remained obscure. Here the plant-specific Networked (NET) superfamily of actin-binding proteins is discussed. Members of this family localize to the actin cytoskeleton and specify different membrane compartments. The founding member of the NET superfamily, NET1A, is anchored at the plasma membrane and predominates at cell junctions, the plasmodesmata. NET1A binds directly to actin filaments via a novel actin-binding domain that defines a superfamily of thirteen Arabidopsis proteins divided into four distinct phylogenetic clades. Members of other clades identify interactions at the tonoplast, nuclear membrane, and pollen tube plasma membrane, emphasizing the role of this superfamily in mediating actin-membrane interactions. This and further work on specific members of the NET superfamily will be discussed.

3.4 TALK 2 – SYMPOSIA 7 – 14:00–14:20

Plant-specific IQD families: novel calmodulin-targets involved in protein recruitment to microtubules

Katharina Bürstenbinder[1], Nadine Schumann[1], Gina Stamm[1], Anshu Khatri[1], Romina Plötner[1], Marcel Quint[1], Steffen Abel[1]

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The IQD (IQ67-domain) family comprises a novel class of plant-specific proteins that interact with calmodulin (CaM) and CaM-Like (CML) Ca2+-sensors. The founding member, Arabidopsis IQD1, stimulates accumulation of defense compounds and resistance against herbivores. IQD1 localizes to the nucle(ol)us and is associated with microtubules (MTs) where it recruits CaM/CMLs and kinesin-light-chain-related-1 (KLCR1), a putative kinesin subunit. Phylogenetic analysis revealed evolution early in the land plant lineage. While absent from green algae, the IQD family already consists of 11 members in the moss *Physcomitrella patens* which cluster in two separate groups indicative of two ancestors. Here, we show that most of the 33 IQD family members of Arabidopsis, as well as select IQD proteins from moss, are associated with MTs, localize to specific sub-nuclear bodies, and differentially interact with the three members of the Arabidopsis KLCR family. Furthermore, analysis of several Arabidopsis lines with altered *IQD* or *KLCR* expression revealed phenotypes related to MT functions such as altered plant morphology and development, or responses to abiotic stimuli, e.g. touch. Taken together, our data suggest that IQD proteins may provide a broad array of MT-associated scaffolds that are possibly involved in Ca2+-dependent regulation of kinesin-dependent intracellular transport.

3.4 TALK 3 – SYMPOSIA 7 – 14:20–14:40

Nuclear phosphoinositides: New roles as regulators of cell cycle, stem cell identity and meristem maintenance?

Katharina Gerth[1], Wilhelm Menzel[1], Stephanie Werner[1], Bettina Hause[2], **Mareike Heilmann**[1]

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Phosphoinositide (PI) signaling has been implicated in the regulation of numerous cellular processes. Studies on yeast and animal cells have shown a distinct additional PI pathway in nuclei, possibly regulating mRNA export, gene transcription, DNA repair or cell cycle progression. It was demonstrated that in animals retinoblastoma (Rb), which regulates the cell cycle by progression of cells from G1 through S phase interacts with type I PIPkinases, enzymes effecting nuclear PtdIns(4,5)P2 synthesis. Plant stem cells in the meristems of shoot and root apices are in a never ending cell cycle and do not differentiate. The loss of the single *Arabidopsis* homolog of Rb, retinoblastoma-related 1 (RBR1), causes reduced cell division rate and delayed differentiation in the root meristem. We have observed that reduced levels of PtdIns(4,5)P2 also result in decreased dividing activity and misshapen stem cell areas in *Arabidopsis* root meristems. So far, the dynamics of nuclear Ptdns(4,5)P2 in relation to cell division events are unclear. By using *Arabidopsis* as a model we are aiming to delineate the effects of altered PI-metabolism on cell cycle control and meristem activity and elucidate open questions.

3.4 TALK 4 – SYMPOSIA 7 – 14:40–15:00

Putative ROP-GAPs are essential for pavement cell shape establishment

Dorothee Stöckle[1], Steffi Zimmermann[1], Sabine Müller[1]

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Small GTPases act as molecular switches in the signal transduction. These small proteins are divided in five subfamilies Ras, Rab, Ran, Arf and Rho GTPases. In plants - Rho GTPases are called Rho of Plants (ROPs) and regulate processes like vesicle trafficking and cytoskeleton organization. Rho proteins are inactivated by GTPase activating proteins (GAPs) by stimulating the intrinsic GTPase activity. *Arabidopsis thaliana* encodes two subfamilies of ROP-GAPs, one of which contains a Pleckstrin homology domain at their N-terminus and is therefore designated PH-GAP subfamily. PH-GAP family includes three closely related members - REN1, PH-GAP1 and PH-GAP2. REN1 was characterized as a regulator of ROP1 activity during polarized pollen tube growth. In our studies we focus on the PH-GAP1 and the PH-GAP2. Double mutants display loss of pavement cell shape complexity which is reminiscent of ROP constitutively active mutants. This observation suggested that PH-GAP1 and PH-GAP2 might regulate ROP2, ROP4 and/or ROP6 activity. In addition we could show that the GAP domain is essential for PH-GAP function.

3.5 TALK 1 – SYMPOSIA 2 – 16:00–16:30

Integrating the signalling networks that trigger programmed cell death in self-incompatible *Papaver* pollen

Noni Franklin-Tong[1]

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Self-incompatibility (SI) is a genetically-controlled mechanism used by many angiosperms to prevent self-fertilization and inbreeding. A multi-allelic *S* locus allows discrimination between "self" (incompatible) pollen from "non-self" pollen on the stigma. Interaction of matching pollen and pistil S-determinants allows "self" recognition and triggers rejection of incompatible pollen. The S-determinants for *Papaver rhoeas* (poppy) are PrsS and PrpS. PrsS is a small novel protein that acts as a signalling ligand that interacts with its cognate pollen S-determinant PrpS, a small novel transmembrane protein. Interaction of PrsS with incompatible pollen stimulates increases in cytosolic free Ca2+ and influx of Ca2+ and K+. ROS and NO signals are also implicated. Downstream targets include the cytoskeleton, a soluble inorganic pyrophosphatase, and a MAP kinase, PrMPK9. The major focus for SI signals is initiation of programmed cell death (PCD). I will provide an overview of our understanding of how PCD in this system operates, focusing on how the signals and components are integrated. I will also discuss our recent functional expression of PrpS in *Arabidopsis thaliana* pollen.

3.5 TALK 2 – SYMPOSIA 2 – 16:30–16:50

Enhanced callose production - an alternative to uncouple pathogen resistance from agricultural tradeoffs

Dorothea Ellinger[1], Bernhard Ellinger[2], Christian A. Voigt[1]

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[2]European ScreeningPort, Hamburg, Germany

Alterations of the cell wall composition or structure that promote pathogen resistance are often accompanied by a negative impact on yield and/or biomass production. Here, we present our recent data from Arabidopsis (*Arabidopsis thaliana*) lines that overexpress the native, constitutively active (CA) or dominant negative (DN) isoform of the GTPase RabA4c. Apart from early and elevated callose production, the non-cellulosic monosaccharide composition of the cell wall was altered after infection with the powdery mildew *Golovinomyces cichoracearum* and during leaf senescence in those Arabidopsis lines that overexpress the native as well as CA isoform of RabA4c. The observed complete powdery mildew penetration resistance in RabA4c overexpressing lines was based on enlarged callose deposits at sites of attempted infection. In addition, quantitative RT-PCR experiments revealed that alterations of pathogen response as well as senescence process correlated with similar transcriptional changes of marker genes highlighting possible interrelations between both processes. Our results help to elucidate the underlying dynamics and propose a new strategy to overcome tradeoffs associated with pathogen resistance.

3.5 TALK 3 – SYMPOSIA 2 – 16:50–17:10

Loss-of-function analysis by tissue-specific inhibition of enzyme activity reveals a role for subtilases in peptide hormone processing and floral organ abscission

Katharina Schardon[1], Lucile Graff[1], Annick Stintzi[1], **Andreas Schaller**[1]

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Subtilisin-like proteases (subtilases, SBTs) have been implicated in the processing of precursor proteins for the generation of peptide hormones as signal molecules in plant defense and development. The confirmation of such a function has been hampered by functional redundancy in the large SBT family. We tackled functional redundancy at the level of enzyme activity by tissue-specific expression of a general SBT inhibitor. In a proof-of-concept experiment, we addressed a potential role for SBTs in abscission. In Arabidopsis, the abscission of floral organs is initiated by a peptide signal derived form a larger precursor protein called IDA, and SBTs are candidate enzymes for IDA processing. Many SBTs are in fact expressed in abscission zones, but there is no abscission defect in any of the single gene knock-outs. However, expression of a general Kazal-type SBT inhibitor in transgenic Arabidopsis plants under control of the *IDA* promoter phenocopied the abscission defect of the *ida* mutant. When these plants were supplied with the active IDA peptide, abscission was rescued and the wild-type phenotype restored. The data indicate that subtilases are required for floral organ abscission and act redundantly in the maturation and activation of IDA. Supporting this notion, one of the SBTs expressed in abscission zones, SBT4.13, was shown to process IDA *in vitro* and upon co-expression in *N. benthamiana*.

3.5 TALK 4 – SYMPOSIA 2 – 17:10–17:30

The deubiquitinating enzyme AMSH and ESCRT-III are required for intracellular trafficking and proper autophagic responses in *Arabidopsis thaliana*

Kamila Kalinowska[1], Anthi Katsiarimpa[1], **Erika Isono**[1]

[1]Technische Universität München, Plant Systems Biology, Freising, Germany

We are interested in the regulation of membrane trafficking by post-translational modification mediated by ubiquitin. Ubiquitination of substrate proteins can be reversed by the action of DUBs (deubiquitinating enzymes), which oppose the activity of E3 ubiquitin ligases. DUBs can thus contribute to the maintenance of free ubiquitin pools and at the same time can also regulate the degradation and stability of a target protein. Associated Molecule with the SH3 domain of STAM (AMSH) belongs to the MPN+ (Jab1/Mov34/Mpr1 Pad1 N-terminal+) domain containing DUB family. We have previously shown that AMSH3 is an essential DUB in Arabidopsis that interacts with core subunits of the ESCRT (Endosomal Sorting Complex Required for Transport)-III. ESCRT-III is required in late stages of endocytosis for membrane scission during cargo sequestration into intraluminal vesicles of MVBs. AMSH1, an AMSH3-related DUB, also interacts directly with thus ESCRT-III and mediates trafficking of ubiquitinated membrane proteins to the vacuole via MVBs. Our physiological and cell biological analyses of *amsh* mutants in Arabidopsis revealed a function of AMSH proteins together with ESCRT-III not only in intracellular trafficking but also in autophagic degradation and hence for the proper autophagic response in Arabidopsis.

4.1 TALK 1 – SYMPOSIA 2 – 16:00–16:30

Setting the stage for stem cell transcription factors

Ming Luan[1], Yujuan Du[1], Hugo Hofhuis[1], Carla Garlinha[1], Marta Laskowski[2], Gabino Sanchez Perez[1], Ben Scheres[1], **Viola Willemsen**[1]

[1]Wageningen University, Plant Developmental Biology, Wageningen, Netherlands
[2]Oberlin college, Oberlin, United States

A set of PLT family members play an important role during the first asymmetric cell divisions in the pericycle founder cells that give rise to lateral root primordia. In *Arabidopsis*, LRP usually do not form adjacent or opposite to one another, and their spacing along the root correlates with its curvature. The first are associated with an elevated auxin response. These divisions require the auxin-responsive protein module SOLITARY-ROOT/IAA14 and ARF7 and ARF19. We will discuss the regulation and the role of PLTs during these early steps of lateral root development. The initial asymmetric division of the plant zygote to give different apical and basal daughter cells has been linked with differential auxin distribution but the underlying mechanism for this generation of cell fate diversity has remained unknown. Expression of the *PLETHORA* genes, encoding AP2 domain containing transcription factors, is detectable from the earliest stages of embryogenesis onward. Overlapping functions of two *PLT* genes promote the asymmetric division of the zygote and trigger development of the apical cell lineage. We will describe two direct upstream regulators of one of these *PLT* genes during the first step of plant embryo development, which both have critical roles in early embryo development. Finally, we discuss possible conserved mechanisms during lateral root initiation early and embryo development.

4.1 TALK 2 – SYMPOSIA 2 – 16:30–16:50

Quantitative and Hormonal control of secondary growth: the *Arabidopsis* hypocotyl as a model

Laura Ragni[1], Kaisa Nieminen[1], Martial Sankar[1], Christian Hardtke[1]

[1]University of Lausanne, DBMV, Lausanne, Switzerland

The *Arabidopsis* hypocotyl has been shown to be a valid genetic model system to study secondary growth (e.g. hypocotyl secondary growth is uncoupled from elongation growth). In the hypocotyl secondary growth proceeds in two phases: an early phase in which xylem and phloem are produced at the same rate by the cambium and a later phase of xylem expansion, in which xylem is produced at higher rate, and fibers differentiate reminiscent of tree stems. Previously, it has been shown that at flowering a shoot-derived signal triggers this xylem expansion (Sibout et al., 2008). We showed that flowering-dependent hypocotyl xylem expansion is a general feature of herbaceous plants with a rosette growth habit. By contrast, in *Arabidopsis* neither flower formation nor elongation of the main inflorescence is required. Recently we have found that the gibberellin (GA), which has been shown to regulate cambial activity and wood deposition in trees, is limiting xylogenesis and that GA signaling is required locally to promote xylem expansion. In addition, the effect of GA was graft-transmittable suggesting that GA is the signaling molecule itself (Ragni et al., 2011). We are currently building a model of secondary development; we will quantify and qualify the cambial growth patterns.

4.1 TALK 3 – SYMPOSIA 2 – 16:50–17:10

Elucidating the gene regulatory network controlling the root stem cell niche

Ernst Aichinger[1], Eric van der Graaf[2], Edwin Groot[1], Thomas Laux[1]

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[2]Institute Plant Sciences, University Graz, Graz, Austria

Plant stem cells are located in apical meristems at the growing tips of the shoot and the root. Within the root meristem, signals from the slowly dividing cells of the so-called quiescent center (QC) maintain the neighboring stem cells in an undifferentiated state. The homeobox gene WUSCHEL-RELATED HOMEOBOX 5 (WOX5) is QC-specifically expressed and plays a central role in the control of the distal stem cells as well as in QC specification. Lack of WOX5 activity results in loss of distal stem cells, QC marker expression and altered QC morphology. To identify the molecular events triggered by WOX5 activity, we performed genome-wide binding and transcriptome profiling studies. First, we identified WOX5 primary target genes by chromatin immunoprecipitation with a WOX5 specific antibody followed by hybridization to a tiling array. Furthermore, the analysis in combination with QC-specific expression data sets allows us to identify direct targets of WOX5 and to describe the molecular mechanisms involved in WOX5-mediated QC specification.

4.1 TALK 4 – SYMPOSIA 2 – 17:10–17:30

Auxin does not affect root growth *per se* and has no regulatory relevance for differential gravitropic growth; - time to say goodbye to a false developmental model?

Hans Georg Edelmann[1]

[1]Universität zu Köln, Biologie und ihre Didaktik, Köln, Germany

From the very beginnings of auxin (IAA)-research, its exogenous application to either intact organs or segments of shoots or roots served for decades as the basis experiment for the elucidation of its growth promoting mechanism. Employing this classical experimental procedure the effect of exogenous IAA on root- and shoot growth was reexamined in maize seedlings. Applied via defined incubation-solutions as well as pastes, IAA promoted elongation growth in coleoptiles. Also, root growth was inhibited in a concentration dependent manner, when roots were incubated in appropriate solutions - a procedure identical to the bulk of previous root growth studies. However, the very same solutions and pastes had no effect on neither elongation growth of roots, nor on gravitropic differential growth, as long as the seedlings were entirely incubated - apart from the root tip/cap; yet, subsequent secondary root formation was strongly affected by IAA. This indicates that the inhibiting effect of IAA on root growth acts via a mechanism originating from and depending on the root cap, mediated by IAA, which itself, however, has no effect on elongation growth of the cells of the root proper. Further physiologically most relevant data will be presented and discussed.

4.2 TALK 1 – SYMPOSIA 3 – 10:30–11:00

The Establishment of Lateral Organ Polarity in Arabidopsis

Marcus Heisler[1], Monica Pia Caggiano[1], Xulian Yu[1], Neha Bhatia[1], Pia Sappl[1], Carolyn Ohno[1]

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Organ formation is a fundamental developmental process in plants and animals. It involves a positioning mechanism that defines where the tissue or organ will arise as well as changes to growth and differentiation that result in morphogenesis and the correct patterning of cell types. By confocal imaging of multiple GFP-labeled proteins we have begun to gain an overall picture of how these interrelated processes are coordinated. The picture that emerges is one ofcross-regulation at multiple levels. For instance, we find that central-peripheral cell-type patterning in the shoot localizes auxin response such that initiating lateral organs are able to inherit their adaxial/abaxialcell type patterning directly from meristem precursor tissue. This is in part mediated by auxin-responsive cell polarity patterns that not only help to further localize auxin via its polar transport, but also regulate morphogenesis at the cellular level via the microtubule cytoskeleton. In turn, localized auxin within the periphery feeds back to cell type patterning which results in changes to the domains of auxin response and future organ development.

4.2 TALK 2 – SYMPOSIA 3 – 11:00–11:20

Evolution of a regulatory module: strigolactones, PIN proteins and shoot branching

Tom Bennett[1], Ottoline Leyser[1]

[1]University of Cambridge, Sainsbury Laboratory, Cambridge, United Kingdom

Strigolactones are a recently identified class of hormone that promote turnover of PIN1 auxin efflux carriers from the plasma membrane, in a fast, direct and MAX2-dependent manner (Shinohara et al, 2013). We have previously proposed that this regulatory module controls shoot branching by limiting canalization of auxin transport between buds and the stem (Prusinkiewicz et al, 2009). Here, we present data that reveal complex patterns of PIN-mediated auxin transport in the stem, and examine the ramifications of this for the canalization model of shoot branching. We examine the universality of the strigolactone-PIN module, and assess whether all PIN proteins are sensitive to strigolactones, and whether all strigolactone responses occur through modulation of auxin transport. Furthermore, we assess how widespread this module might be in land plants, drawing on phylogenetic analyses including a complete reassessment of evolution of the PIN protein family, and experiments in the moss *Physcomitrella patens*. We conclude that the strigolactone-PIN module may be ancient, but is only part of a complex MAX2-depedent regulatory network.

4.2 TALK 3 – SYMPOSIA 3 – 11:20–11:40

CLE peptide signaling inhibits cambium activity in *Arabidopsis* in a MOL1-dependent fashion

Nial Gursanscky[1], Javier Agusti[2], Karin Grünwald[1], Thomas Greb[1]

[1]Gregor Mendel Institute for Molecular Plant Biology, Vienna, Austria
[2]University of Oxford, Department of Plant Sciences, Oxford, United Kingdom

Question: We recently identified the leucine-rich repeat receptor-like kinase (LRR-RLK) MORE LATERAL GROWTH 1 (MOL1) that inhibits cambium activity in *Arabidopsis*. MOL1 has a putative CLE-peptide binding site with high similarity to that in CLV1, an LRR-RLK active in shoot apical meristems. In this study we aim to identify the putative ligand of the MOL1 receptor and elucidate the role of MOL1 in cambium regulation. Methods and Results: *MOL1* promoter-reporters show that *MOL1* is expressed in phloem cells abaxially to the cambium. We tested candidate CLE peptides for their cambium regulating activity and found that one CLE peptide specifically reduces cambium activity in a *MOL1*-dependent manner. Interestingly, promoter-swapping experiments show that *MOL1* can partially replace *CLV1* in the shoot apical meristem. Furthermore, *CLE41*, which promotes cambium activity and is expressed in the phloem, is more active in *mol1* mutants. Conclusions: We conclude that *MOL1* acts in the cambium in a similar fashion as *CLV1* in shoot apical meristems. Like *CLV1*, *MOL1* represses meristem activity depending on the activity of a CLE signaling peptide. We propose that the non-cell autonomous effect on cambium activity downstream of *MOL1* depends on reducing the levels of mobile CLE41 peptide in the phloem. Collectively, our analyses reveal a strong similarity in the regulation of apical and lateral plant meristems.

4.2 TALK 4 – SYMPOSIA 3 – 11:40–12:00

Interaction of Phytochrome A and SPA1 promotes photomorphogenesis in far-red light

David Sheerin[1], Chiara Menon[1,2], Sven zur Oven-Krockhaus[2], Philipp Johnen[2], Frank Schleifenbaum[2], York-Dieter Stierhof[2], Andreas Hiltbrunner[1,3]

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[2]University of Tübingen, Centre for Plant Molecular Biology, Tübingen, Germany
[3]BIOSS Centre for Biological Signalling Studies, Freiburg, Germany

Phytochromes function as red/far-red photoreceptors in plants, and are essential for light-regulated growth and development. Depending on the light conditions, plants follow different developmental programs. Photomorphogenesis, the developmental program in light, is the default program in seed plants. In dark-grown seedlings, photomorphogenic growth is actively suppressed by the action of the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1)/SUPPRESSOR OF *phyA-105* (SPA) complex, which targets positive regulators of photomorphogenic growth for degradation by the proteasome. Phytochrome A (PHYA) is essential for seedling establishment in canopy shade, where the light environment is dominated by far-red light. It is well established that light-activated PHYA inhibits the COP1/SPA complex leading to the accumulation of transcription factors promoting photomorphogenesis; yet, the mechanism by which PHYA inactivates COP1/SPA is still unknown. We identified SPA1 as an interactor of light-activated PHYA in a yeast two-hybrid screen. FRET-FLIM analyses confirm that SPA1 and PHYA co-localise and interact *in planta*. Moreover, we demonstrate that PHYA competes with COP1 for binding to SPA1, providing a molecular mechanism for the light-induced inactivation of the COP1/SPA complex by PHYA.

Session 4.3 – Reproductive Development I: Gametophyte and Fertilization

Tuesday 10/01 – 14:00-15:30 – Chair: Ueli Grossniklaus

4.3 TALK 1 – SYMPOSIA 4 – 14:00–14:30

Molecular Control of Fertilization and Interspecific Hybridization

Lena Müller[1], Heike Lindner[1], Sharon A. Kessler[1], Michael T. Raissig[1], Hiroko Shimosato-Asano[1], **Ueli Grossniklaus**[1]

[1]University of Zurich, Institute of Plant Biology, Zurich, Switzerland

We have isolated and characterized female gametophytic mutants that disrupt pollen tube reception. Pollen tubes that encounter such mutant female gametophytes are unable to rupture and release the sperm cells (Huck et al., *Development* 130:2149; Kessler et al., *Science* 330:968). These phenotypes suggest that the female gametophyte controls the behaviour of the male gametophyte (pollen) in this process. One of the mutants, *feronia,* was shown to affect a receptor-like kinase (Escobar-Restrepo et al., *Science* 317:656), while another, *nortia*, disrupts a seven-transmembrane-domain-protein similar to the powdery mildew resistance protein Mlo (Kessler et al., *Science* 330:968). The identification of additional components in this signal transduction cascade suggest the involvement of glycosylation in this recognition process. Furthermore, interspecific crosses between Brassicaceae can result in a similar phenotype, suggesting the cell-cell interactions during pollen tube reception may be involved in interspecific crossing barriers. Using genome-wide association studies, we have been able to identify a factor that plays a specific role in interspecific compatibility while intraspecific crosses are not affected. Thus, pollen tube reception may be involved in establishing crossing barriers essential to maintain species boundaries similar to sperm-egg interactions in animals.

4.3 TALK 2 – SYMPOSIA 4 – 14:30–14:50

From guard to guide: Evolutionary functional diversification of defensins in *Arabidopsis*.

Mariana Mondragón Palomino[1], Ajay John-Arputharaj[1], Thomas Dresselhaus[1]

[1]University of Regensburg, Cell Biology and Plant Biochemistry, Regensburg, Germany

Defensins and defensin-like proteins (DEFLs) are part of a large, ancient and diverse family of cysteine-rich peptides involved in the first line of defense against bacteria and fungi. In plants, several species-specific DEFLs also play a key role in the communication between male and female gametophyte during fertilization by mediating pollen tube guidance and burst. Here I present work aiming to understand how in the course of diversification of the genus *Arabidopsis* distinct DEFLs became involved in the establishment of species-specific signals to mediate gametophyte communication during fertilization. Specifically, I introduce the phylogeny, patterns of gene loss and gain as well as the relationships of orthology of all DEFLs from *Arabidopsis thaliana*, *Arabidopsis halleri* and *Arabidopsis lyrata*. Based on this evolutionary framework, I discuss the correspondence between specific clades with both functional information and patterns of expression measured by RNA-seq during fertilization and in response to pathogenic fungus *Fusarium graminearum*. In this context, I consider the processes driving the evolution of DEFLs by reviewing information on the regimes of natural selection characterizing each clade of DEFL genes.

4.3 TALK 3 – SYMPOSIA 4 – 14:50–15:10

The role of heat stress transcription factor HsfA2 in vegetative and reproductive tissue development and heat stress response in tomato (*Solanum lycopersicum*)

Sotirios Fragkostefanakis[1], Stefan Simm[1,2], Kerstin Pohl[1], Puneet Paul[1], Enrico Schleiff[1,2], Klaus-Dieter Scharf[1]

[1]Goethe University, Institute for Molecular Biosciences, Frankfurt a.M., Germany
[2]Goethe Univesity, Cluster of excellence, Frankfurt a.M., Germany

Heat stress transcription factors (HSFs) trigger the transcriptional activation and rapid accumulation of heat shock proteins to compensate for the disturbed proteostasis under adverse stress conditions. Tomato genome comprises 24 HSF genes but major aspects of heat stress response (HSR) and recovery are regulated by the interaction of HsfA1a with HsfA2 and HsfB1. Using a bioinformatics co-expression approach we identified a putative regulatory network for HsfA2 with potential role in abiotic stress responses. HsfA2 is expressed at basal levels in vegetative tissues under non-stress conditions but is strongly induced in early stages of pollen development indicating possible developmental function. To investigate the role of HsfA2 in plant development and HSR, we used tomato lines (*S. lycopersicum* cv Moneymaker) transformed with expression cassettes encoding HsfA2 in sense and antisense orientation either causing its constitutive expression or RNAi-mediated knock-down, respectively. Phenotypic analyses showed that HsfA2 is involved in several developmental aspects including pollen quality. To get more insights into the role of HsfA2 in both vegetative and reproductive tissues, we performed a transcriptome analysis using Next Generation Sequencing of control and heat stressed leaves and anthers. Our results show major differences between the HSR in vegetative and male reproductive tissues and point to the direction of HsfA2 as an important transcriptional regulator of many genes related with thermotolerance.

4.3 TALK 4 – SYMPOSIA 4 – 15:10–15:30

The plastid-localized NAD-dependent malate dehydrogenase is crucial for energy homeostasis in developing *Arabidopsis thaliana* seeds

Jennifer Selinski[1], Nicolas König[1], Benedikt Wellmeyer[1], Guy T. Hanke[1], Vera Linke[1], H. Ekkehard Neuhaus[2], Renate Scheibe[1]

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In the absence of photosynthesis, ATP is imported into chloroplasts and non-green plastids by ATP/ADP transporters or formed during glycolysis, the latter requiring continuous regeneration of NAD+, supplied by the plastidial isoform of NAD-MDH. During analysis of T-DNA insertion mutants of *A. thaliana* only heterozygous but no homozygous mutants could be identified. These heterozygous plants show higher transcript levels of an alternative NAD+-regenerating enzyme, NADH-GOGAT, and, remarkably, improved growth when ammonium is the sole N-source. In-situ hybridization and GUS-histochemical staining revealed that plNAD-MDH was particularly abundant in male and female gametophytes. A knockout of plNAD-MDH has a strong effect on pollen tube growth. Knock-out pollen lacking plNAD-MDH do not germinate in vitro, but can fertilize the egg cell in vivo. However, young siliques of selfed heterozygous plants contain both green and white seeds corresponding to wild-type/heterozygous (green) and homozygous knock-out (white) mutants in a (1:2):1 ratio. Embryos of the knock-out seeds only reached the globular stage, did not green, and developed to tiny wrinkled seeds, suggesting that a blocked major physiological process in *plNAD-MDH* mutants stops both, embryo and endosperm development in order to avoid assimilate investment in compromised offspring.

4.4 TALK 1 – SYMPOSIA 7 – 13:30–14:00

The Developmental History of a Fruit

Lars Ostergaard[1]

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Multicellular organisms such as animals and plants develop specialised organs, which are composed of different types of tissues. The structure - or pattern - of organs is determined by the polarity within tissues. Interactions among key regulators during *Arabidopsis* gynoecium development have revealed a network of upstream transcription factor activities required for dividing this organ into discrete domains. Dynamics of the plant hormone auxin is emerging as an immediate downstream output from these activities, and here we aim to understand the spatiotemporal information that is defined through interactions between a set of transcription factors and auxin during the gynoecium patterning process. At the whole-organ level we propose that differentially oriented auxin flows define polarity and position of tissue boundaries along the apical-basal axis. At a local scale, our data suggest that auxin is recruited to provide uniform identity to cells in the apical region of the gynoecium to ensure radial symmetry of the style tissue. We hypothesise that this is required to eliminate a default state of medio-lateral symmetry engrained in the gynoecium due to its origin as two fused leaves. An emerging feedback regulatory mechanism for gynoecium development will be presented in which auxin-controlled transcription factor interactions may determine the identity of downstream targets.

4.4 TALK 2 – SYMPOSIA 7 – 14:00–14:20

Is trehalose-6-phosphate (T6P) a general signal gating developmental transitions?

Vanessa Wahl[1], Magdalena Dzialo[1], Armin Schlereth[1], Jathish Ponnu[2], Markus Schmid[2], Mark Stitt[1]

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T6P has been implicated as a signal of sucrose status (1), thereby serving as a link between developmental processes and the metabolic and energy status of the plant. However, the molecular mechanism by which this signal is integrated into the complex developmental networks is not well understood. We have recently shown that T6P plays a central role in the induction of flowering acting in the leaves via the *FT* node of the photoperiod pathway and at the shoot apex (SAM) via the age pathway (2). T6P is synthesized from G6P and UDPG by the catalytic activity of TPS1. *TPS1* is expressed in vascular tissue throughout a plant's life, but it also shows a distinct expression domain in the peripheral zone of the SAM from the heart stage of embryogenesis until right before the transition to flowering. A homozygous T-DNA insertion line is embryo lethal, with arrested embryos at the early torpedo stage, which is exactly when sucrose levels rise (3). *35S::amiRTPS1* plants with reduced levels of *TPS1* and T6P are late flowering but are still able to set viable seeds. These plants have significantly more juvenile leaves, arguing for a delay in the vegetative phase change. In summary, previous observations and our own data already suggest that T6P is crucial for the correct timing of developmental transitions in general.

1) Lunn et al., Biochemical Journal, 2006

2) Wahl et al., Science, 2013

3) Eastmond et al., Plant Journal, 2002

4.4 TALK 3 – SYMPOSIA 7 – 14:20–14:40

Restoration of lateral spikelet fertility in two-rowed barley by RNA-interference of *Vrs1*

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Barley (*Hordeum vulgare*) spikes are developmentally switched from two-rowed to six-rowed by a single recessive gene, *six-rowed spike 1* (*vrs1*), which encodes a homeodomain-leucine zipper I class transcription factor located on the long arm of chromosome 2H [1]. *Vrs1* is a paralog of *HvHox2* and both were generated by duplication of an ancestral gene. *HvHox2* is conserved among cereals, whereas *Vrs1* acquired its current function during the evolution of barley [2]. Loss of function of *Vrs1* results in six-rowed spikes by restoring fertility of the lateral spikelets. A six-rowed cultivar, Hayakiso-2 showed significantly reduced *Vrs1* expression suggesting a quantitative manner of *Vrs1*. To demonstrate the quantitative function of *Vrs1,* we transformed the two-rowed barley cv. 'Golden Promise' with a *Vrs1*-specific hairpin-RNA interference (RNAi) construct under the control of the rice *ACTIN1* promoter or the doubled enhanced *CaMV 35S* promoter. Transgenic plants with reduced level of *Vrs1* expression exhibited an elongated awn in the lateral spikelets and some plants produced grains, depending on the promoter strength driving the expression of the RNAi cassette. Our data provide compelling evidence that *Vrs1* expression suppresses the development of lateral spikelets in a quantitative manner.

[1] Komatsuda *et al* (2007) Proc Natl Acad Sci USA **104**:1424-1429

[2] Sakuma *et al* (2013) New Phytol **197**:939-948

4.4 TALK 4 – SYMPOSIA 7 – 14:40–15:00

The Role of Oligo Fructans During Barley Grain Development

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Cereal seeds exhibit a pivotal role for animal and human nutrition. Thus, the processes involved in nutrient import and storage product accumulation are of utmost interest. Mass spectrometry based imaging analyses of metabolites during barley grain development revealed highly specific features for the nutrient transfer region that were taken for targeted analyses on metabolite, transcript and protein level. Particular patterns for oligo fructans were found with the branched type fructan bifurcose produced prior to the onset of storage accumulation and the inulin type fructans (1-kestose and nystose) concentrating around the endospermal cavity during the storage stage. Corresponding patterns were observed by qRT-PCR of micro-dissected seed material for genes from fructan metabolism. The branched form apparently relates to a transient storage. The linear type fructans are suggested to be involved in mediation of stress responses such as membrane stabilization and detoxification of reactive oxygen species (ROS) as the vitality of the grain region directing the massive nutrient import has to be maintained until storage product accumulation has finished. The tissues of the nutrient transfer region are characterized by high transport activities and programmed cell death, both processes producing ROS. *In vitro* experiments coincide with the hypothesized protective function.

4.5 TALK 1 – SYMPOSIA 9 – 10:30–11:00

Novel concepts in *Arabidopsis* shoot meristem stem cell maintenance

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In the shoot apical meristem, a population of pluripotent stem cells gives rise to all aerial organs throughout the plant's life, which in extreme cases, such as trees, can last for centuries. Although much is known about the maintenance of the stem cell pool, many processes are poorly understood. Forward genetic mutant screens in *Arabidopsis* provided only a limited number of loci involved in stem cell regulation, possibly due to genetic redundancy. To circumvent this problem, we performed several screens for genetic modifiers in sensitized background. We identified a set of genes not previously implied in stem cell regulation, making it possible to study functions previously hidden by genetic redundancy. Novel insights into the principles of stem cell regulation provided by the functional study of these genes will be discussed. We gratefully acknowledge funding from the Deutsche Forschungsgemeinschaft (ERA-PG, SFB592) and the Interreg IV program (Trinational Institute of Plant Sciences).

4.5 TALK 2 – SYMPOSIA 9 – 11:00–11:20

The green hourglass - a phylotranscriptomic comparison of plant and animal embryogenesis

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Animal and plant embryogenesis evolved independently in both lineages. Comparative anatomy of vertebrate development — based on von Baer's laws of embryology from the early nineteenth century — shows that embryos from various taxa appear different in early stages, converge to a similar form during mid-embryogenesis, and again diverge in later stages. This morphogenetic series is known as the developmental 'hourglass', and its bottleneck of high conservation in mid-embryogenesis is referred to as the phylotypic stage. Although extensively explored in animals, a developmental hourglass has not been reported in plants, which represent the second major kingdom in the tree of life that evolved embryogenesis. Here we provide phylotranscriptomic evidence for a molecular hourglass in *Arabidopsis thaliana* embryogenesis. Furthermore, the apparent absence of a morphological hourglass in plants suggests that morphological and molecular patterns might be uncoupled. These findings indicate convergent evolution of the molecular hourglass and a conserved logic of embryogenesis across kingdoms. Lastly, we provide additional data which suggest that developmental processes other than embryogenesis might also be regulated by evolutionary conserved transcriptional patterns.

4.5 TALK 3 – SYMPOSIA 9 – 11:20–11:40

Mutations in *RPK1* uncouple formation of cotyledon anlagen and primordia growth in *Arabidopsis thaliana* embryos by modulating epidermal cell shape and polarity.

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Mutants in the *Arabidopsis* receptor-like kinase RPK1 omit one cotyledon with low penetrance due to complex genetic redundancy. However, *RPK1´s* link to organisers of cotyledon initiation and development such as the Ser/Thr kinase *PID*, the NPH3-like protein *ENP* and the auxin-efflux carrier *PIN1* is unclear. We have analysed expression of *PID*, *ENP* and PIN1 as well as the primordia initiation marker *ANT* in embryos carrying the strong fast neutron allele *rpk1-7*. We have also analysed expression of genes controlling shoot apical meristem (SAM) organisation. The results demonstrate that cotyledon anlagen but not primordia are perfectly elaborated in *rpk1*. Our data support the view that the reduction in cotyledon number is caused by epidermis specific alterations affecting cell shape and polarity in a timely and spatially stochastic fashion in *rpk1* embryos. This dysfunction can only manifest after activity of PID and ENP disturbing the fidelity of the cellular machinery, which establishes and stabilises convergence points and auxin maxima respectively. Since auxin maxima induce organ primordia, *rpk1* effects explain how monocotyledonous seedlings and variants of cotyledon shape can developmentally arise in *Arabidopsis* and perhaps in other plants.

4.5 TALK 4 – SYMPOSIA 9 – 11:40–12:00

Overexpression of embryogenesis- and growth-related genes in transgenic *Pinus pinaster* embryos

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Our intention was to study the effect of overexpressing embryogenesis- (*PpLEC1*, *PpWOX2*) and growth-related genes (*PpSUSY*, *PpASPG*) in *Pinus pinaster* during somatic embryogenesis. Transgenic (PCR positive) lines were obtained through *Agrobacterium*-mediated transformation of embryogenic tissue. Both *CaMV35S* and *UBI-1* constitutive promoters resulted in similar and acceptable reporter gene activity (*GUS* or *GFP*) in transgenic tissue. Hence, *PpLEC1* and *PpWOX2* were cloned into a binary vector driven by the *CaMV* 35S promoter whereas *PpSUSY* and *PpASPG* were driven by the *UBI-1* promoter. T-DNA integration into embryogenic tissue and regenerated embryos was confirmed by PCR. Morphological studies indicated that somatic embryos of *PpLEC1* transgenic lines were arrested at filamentous stage. Germination ability of mature embryos was highly variable between lines. Root formation was reduced significantly for most *PpASPG* transgenic lines compared to non-transformed control. qRT-PCR analysis showed an up-regulation of *PpLEC1* and *PpSUSY* in transgenic lines at early embryogenic stages whereas *PpWOX2* and *PpASPG* was only up-regulated in several transgenic lines at late embryogenic stages. Spatio-temporal regulation of transgenes' expression during embryo development could be due to significant fluctuations in expression of the respective genes, already seen in wild type embryogenesis.

5.1 TALK 1 – SYMPOSIA 1 – 14:00–14:30

GWA reveals complex adaptive history for glucosinolate profiles in *A. thaliana*

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Plant populations exhibit extensive variability in resistance traits. Understanding how natural selection shapes this variability can aid efforts to improve conservation and agriculture. The "mustard oil bomb" characterized by blends of glucosinolates is a key defense against herbivory and pathogenic attack in many plant species. We conducted a GWA study of 22 methionine-derived glucosinolates in leaves of 757 *Arabidopsis thaliana* accessions. We detect several known and novel candidates. If selection acts on this complex trait, genome-wide signatures may be created. Genome scans for Fst revealed 240-fold enrichment of SNPs underlying variation in glucosinolates. This suggests a strong adaptive differentiation of loci underlying glucosinolate natural variation. Additionally, we observed continental-scale longitudinal clines in the allele frequency of two epistatic loci crucial for the production of C4 hydroxy-alkyl/alkenyl glucosinolates. Although located on different chromosomes, the two loci displayed strong, genome wide significant correlation, suggestive of epistatic selection. Combined with fitness estimates and scoring of herbivore damage in field experiments within the species range, our results indicate a strong adaptive role of loci underlying natural variation of glucosinolate profiles and their epistatic interactions.

5.1 TALK 2 – SYMPOSIA 1 – 14:30–14:50

Non-random distribution of autoimmunity loci in *Arabidopsis thaliana*

Eunyoung Chae[1], Sang-Tae Kim[1], Darya Karelina[1], Maricris Zaidem[1], Carmen Martin Pizarro[1], Kirsten Bomblies[1], Detlef Weigel[1]

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Autoimmune responses are commonly observed phenomena in inter- and intra-specific hybrids of plant species. Genetic variation of plant immune receptors often associates with this autoimmunity in hybrids. To understand fitness cost of immune system variation and molecular mechanisms of autoimmunity, we generated diallel crosses of 80 genomes of *A. thaliana*. Screening of F1 hybrids for autoimmunity followed by QTL analyses using restriction site associated DNA sequencing led us to conclude that the distribution of the causal loci is highly non-random. Most causal loci overlap with NB-LRR dense regions of genomes. The *RPP1/DM2* locus emerges as a major contributor to the autoimmunity, explaining five independent cases of which causal alleles are widespread in populations. Extensive variations in sequence, copy number and genomic structure are the key features of *DM2*. The identification of the two casual *DM2* genes shows that they underwent heterogeneous evolutionary processes to generate the causal alleles. The five partners of the causal DM2s are distinct in genome location and types of protein. Especially, the DM2d-DM1 partnership is characterized by two interacting TIR-NB-LRRs, both of which enzymatic activities are required for the hypersensitive response in a heterologous system. With pairs of autoimmunity proteins, we can investigate how the immune receptors are activated.

5.1 TALK 3 – SYMPOSIA 1 – 14:50–15:10

Evolution of homospermidine synthase in the Convolvulaceae - a story of gene duplication, gene loss, and periods of various selection pressures

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Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, is known to have its origin in the duplication of a gene encoding deoxyhypusine synthase. To study the processes that followed this duplication event and gave rise to homospermidine synthase, we have identified sequences encoding homospermidine synthase and deoxyhypusine synthase from various species of the Convolvulaceae. We show that one ancient duplication of deoxyhypusine synthase occurred in this family, which was followed by gene loss and pseudogenization of one copy in several lineages. In those lineages in which the gene copy was successfully recruited as homospermidine synthase, statistical analyses of sequence data suggest that varying selective constraints, including purifying, relaxed and possibly positive Darwinian selection, fixed and shaped the new gene. Site-specific mutagenesis experiments have confirmed that the substitution of sites predicted to be under positive Darwinian selection is sufficient to convert a deoxyhypusine synthase into a homospermidine synthase. In addition, analyses of transcript levels show that homospermidine synthase and deoxyhypusine synthase also diverged with respect to their regulation. We suggest that protein-protein interactions affected strongly the evolutionary fate of the duplicated deoxyhypusine synthase gene.

5.1 TALK 4 – SYMPOSIA 1 – 15:10–15:30

Connecting ecology, evolution and molecular biology in species without sequenced genomes with RNA-seq

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Plant survival in marginal habitats such as dry, hot savannahs depends on the evolution of biochemical and anatomical adaptations at the cellular and tissue level. Traits such as C4 photosynthesis, waxy leaf coats and exposure minimizing leaf shapes among many others have evolved convergently in multiple habitats. RNA-seq, the sequencing of the transcriptome by next generation sequencing, provides information about both gene sequence and quantitative gene expression which underly the macroscopic traits. I will use C4 photosynthesis as a case study to demonstrate how RNA-seq identifies genes and pathways required in convergently evolved traits. To date nine species pairs differing for the trait of interest were RNA-seq'ed with 454 Roche technology and/or Illumina technology. The strategies and exemplary results for read assembly will be presented. The quantitative gene expression data has identified both candidate pathways and candidate genes relevant to C4 photosynthetic evolution and function. To illustrate the molecular and evolutionary potential of RNA-seq to analyze species without sequenced genomes, a case study of pyruvate transport function will be presented.

5.2 TALK 1 – SYMPOSIA 4 – 14:00–14:30

QTL analysis of natural variation: Dissecting the regulatory circuitry underlying quantitative traits

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Natural variation can be observed for many traits in nature and it is a long-standing question why variation is maintained over evolutionary time scales. A first step in addressing this issue is the identification of the causal genetic factors. Most of the natural variation is controlled by polygenic regulatory networks with often small effects of contributing components. The identification of the genes involved in the control of these so-called quantitative traits is not trivial. In the past, numerous studies have shown the usefulness of experimental mapping populations for detecting quantitative trait loci (QTLs). More recently, natural populations have come in use for high-resolution genome wide association (GWA) mapping. Both approaches have yielded valuable information about the complex regulation of many traits but suffer from inherent detection bias. We will show the complementary use of both approaches and discuss the implications of our findings in an evolutionary context.

5.2 TALK 2 – SYMPOSIA 4 – 14:30–14:50

Natural variation in the *Arabidopsis* circadian clockwork

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The circadian clock allows plant and animals to anticipate upcoming environmental changes and to mount an appropriately timed response. In the model plant *Arabidopsis thaliana*, several interlocked loops control the expression of ~35% genes and contribute to plant fitness. We performed a search for natural dominant modifiers of clock function by crossing a reporter construct (*TOC1:LUC*) in the Col-2 background to 100 accessions. A single accession, Lö-1, increased *TOC1:LUC* amplitude 4-5 fold over controls, while maintaining a 24-hr period and a normal phase. Other circadian reporters showed normal amplitude when crossed to Lö-1, indicating a specific effect on the *TOC1* locus. A *TOC1* promoter resection series revealed that the high amplitude phenotype was mediated by the 5′ UTR. Endogenous *TOC1* mRNA levels were not affected in F1 plants, suggesting that the Lö-1 modifier acts at the post-transcriptional level. The *TOC1* 5′ UTR contains 2 potential upstream open reading frames (uorf), one being conserved in *A. thaliana* relatives. We hypothesize that a uORF may play a role in controlling *TOC1* translation: to test this, we have generated new *TOC1:LUC* reporters whereby each uORF ATG has been mutated. The high amplitude phenotype behaves as a single semi-dominant locus, which we have mapped to ~ 30 kbp on the upper arm of chromosome 3, away from known clock genes.

5.2 TALK 3 – SYMPOSIA 4 – 14:50–15:10

Evolution of C4 photosynthesis in the genus *Flaveria* - diurnal changes of the leaf transcriptomes of C3, C3-C4 and C4 species

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C4 photosynthesis has evolved multiple times independently in many land plant families. While the basic biochemistry of the C4 cycle is well understood, our knowledge on how this cycle and the overall photosynthetic metabolism is regulated is quite poor. So far no transcription factors and only few post-transcriptional regulators of the C4 genes are known. It is also not clear how many of these regulatory genes must have been altered during C4 evolution and how they were altered. To gain insight into the extent to which gene expression patterns were altered in the evolutionary progression from C3 to C4 leaves we have carried out a comparative transcriptome analysis of leaves of closely related C3, C4 and C3-C4 intermediate species of the genus *Flaveria*. Thereby we profiled leaf transcriptomes via Illumina sequencing of mRNAs (RNA-Seq) during a 24 hour day and night cycle taking samples every four hours. During the analyses we concentrate on genes related to photosynthetic metabolism and the C4 pathway and identify candidates for regulatory components with similar diurnal expression patterns. Because C4 and C3 photosynthesis differ in their regulatory requirements, the comparison of the diurnal changes in gene expression of closely related C3/C4 species pairs allows the identification of putative metabolism regulators that changed during C4 evolution.

5.2 TALK 4 – SYMPOSIA 4 – 15:10–15:30

A new subfamily of cytokinin receptors is revealed by an analysis of the evolution of the two-component signaling system of plants

Gruhn Nijuscha[1], Mhyeddeen Halawa[1], Berend Snel[2], Michael Seidl[2], **Alexander Heyl**[1]

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The two-component signaling system (TCS) - the major signaling pathway of bacteria - is found among higher eukaryotes only in plants where it regulates diverse processes such as the signaling of the phytohormones cytokinin and ethylene. Cytokinin is detected by a hybrid-histidine kinase receptor and the signal is transduced by a multi-step phospho-relay system of histidine phosphotransfer proteins (HPT) and different classes of response regulators (RR). To shed light on the origin and evolution of TCS members in plants, we conducted a comprehensive domain-based phylogenetic study across the relevant kingdoms including charophyceae algae, the group of green algae giving rise to land plants. Surprisingly, we identified and then experimentally characterized a novel subfamily of cytokinin receptors. Moreover, we provide first evidence for cytokinin receptors in the charophyceae *Spirogyra pratensis*. HTPs of charophyceae seemed to be more closely related to those of land plants than to other groups of green algae. Further down the signaling pathway, the type-B RRs were found across all plant clades, but remarkably many members lack either the canonical Asp residue or the DNA-binding domain. Finally, the analysis provides evidence that one additional group of RRs, the type-C RRs, might be degenerated receptors rather than *bona fide* response regulators.

5.3 TALK 1 – SYMPOSIA 5 – 16:00–16:30

Gene duplication as a mechanism for generating novel floral organ identity.

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Across the angiosperms there are many examples of independently derived, novel floral organs. The presence of such structures results in five or more distinct floral organ identities, which is difficult to reconcile with the canonical ABC model. In the emerging model system *Aquilegia* there are five differentiated floral organ types: petaloid sepals, spurred petals, stamens, staminodia and carpels. This morphology would seem to require modifications of the ABC model, namely the capacity to specify two types of petaloid organs as well as a fifth organ identity. Detailed expression and RNAi studies of the three *APETALA3* (*AP3*) paralogs and one *PISTILLATA* (*PI*) homolog in *Aquilegia* demonstrate that each organ type expresses a specific combination of genes. We have now extended this study to include homologs of the C function gene *AGAMOUS*, of which there are two copies in *Aquilegia* (*AqAG1* and *AqAG2*). Analysis of the functions of these genes using transient RNAi reveals complex functional repertoires and evidence for biochemical differentiation between the paralogs. Our refined *Aquilegia* ABC model reveals that through gene duplication, pre-existing floral organ identity programs can be partitioned and modified to produce additional organ types via both sub- and neofunctionalization.

5.3 TALK 2 – SYMPOSIA 5 – 16:30–16:50

Evolution of a novel flower trait in the Brassicaceae

Andrea Busch[1], Stefanie Horn[1], Sabine Zachgo[1]

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A relevant step in angiosperm radiation was the adaptation to pollinators via the establishment of flower monosymmetry, which evolved repeatedly in different angiosperm lineages. In all taxa analysed so far, monosymmetry development is controlled by TCP transcription factors. The Brassicaceae are dominated by polysymmetric species and only six genera form flowers with two petal pairs of different sizes, making it an ideal system to study monosymmetry evolution.

In *Iberis amara*, the first monosymmetric crucifer analysed, unequal petal pair formation is due to a stronger expression of *IaTCP1* in the smaller, adaxial petals. We show that this is also the case for additional *Iberis* species and two other monosymmetric crucifer genera. A phylogenetic reconstruction of the crucifers places all monosymmetric species in one clade. Analyses of the early and late expression of *TCP1* orthologs in mono- and polysymmetric members, which are representative of the four main crucifer lineages demonstrate that crucifer monosymmetry evolved via a heterochronic expression shift from an early adaxial expression in floral meristems in polysymmetric species to a late expression in adaxial petals in monosymmetric members. Via RNAseq and *Arabidopsis microarray* technology we aim to get an overview of the *I. amara* petal transcriptome and to gain insight into the molecular network controlled by *IaTCP1*.

5.3 TALK 3 – SYMPOSIA 5 – 16:50–17:10

Molecular Dissection of the Selfing Syndrome in the Genus *Capsella*

Anahid Powell[1], Michael Lenhard[1]

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The transition from outcrossing to selfing occurs frequently in the evolution of flowering plants and is commonly accompanied by a suite of morphological changes to the flower termed the selfing syndrome. These changes include reduced flower size and opening. A speciation event within the genus *Capsella* led to the divergence of the self-compatible *C. rubella* lineage from the self-incompatible *C. grandiflora*. *C. rubella* reproduces predominantly by self-fertilization and shows several characteristics associated with the selfing syndrome. In contrast, *C. grandiflora* shows large flowers adapted for attracting animal pollinators. These two species were used to create recombinant inbred and near-isogenic lines, which, combined with the genome availability of *C. rubella,* created an attractive model system in which to dissect the molecular basis for the evolution of the selfing syndrome. In particular petal size and petal opening angle have been mapped using these lines. Both traits proved to be complex, but with a limited number of loci showing major effect. By QTL mapping two of these loci, I seek to identify the molecular changes responsible for the reduced petal size and opening angle observed in *C. rubella*. Further localization of the changes responsible for the alternate floral phenotypes will shed light on the mechanism of morphological evolution in floral traits associated with pollination, as well as provide information about the regulation of discrete organ size and the control of petal movements at the molecular level.

5.3 TALK 4 – SYMPOSIA 5 – 17:10–17:30

Shedding Light on the Evolutionary Conservation of Phytochrome Signalling in Land Plants

Anja Possart[1,2], Tengfei Xu[1], **Andreas Hiltbrunner**[1,3]

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Phytochromes (PHYs) are red/far-red light receptors important for plant development. In seed plants, light-activated PHYs translocate into the nucleus, where they interact with PHY INTERACTING FACTORS (PIFs) to regulate gene expression. Responses to high intensity far-red light, so called high irradiance responses (HIRs), allow plants to establish under shade conditions. HIRs require both phytochrome A (PHYA) and FAR-RED ELONGATED HYPOCOTYL 1 (FHY1), a protein essential for PHYA nuclear transport, and have been considered unique to seed plants because the divergence of seed plants and cryptogams preceded the evolution of PHYA. However, we identified HIR-like responses in the moss *Physcomitrella patens*, which depend on ppFHY1, a functional homolog of seed plant FHY1. We also demonstrate that *Physcomitrella* PHY1 is rapidly degraded in light and requires ppFHY1 for nuclear accumulation, suggesting that ppPHY1 and its closest homolog ppPHY3 mediate the HIR-like responses in *Physcomitrella*. In seed plants, PIFs are important PHY downstream signalling components that have different motifs for binding to PHYA and PHYB. We identified PIF-like proteins in *Physcomitrella* (ppPIFs) and show that they bind to ppPHY1 using the motif essential for interaction with PHYA. Interestingly, expression of ppPIFs in *Arabidopsis* results in a phenotype reminiscent of PIF5 overexpressing lines.

5.4 TALK 1 – SYMPOSIA 3 – 10:30–11:00

From pots to plots to continents - functional biodiversity research and functional biogeography in a data-rich world

Christian Wirth[1]

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Functional biodiversity research within the plant sciences aims at elucidating the role of plant biodiversity and identity in controlling ecosystem functioning. Early work in the 1990`s established experiments that kept the environment constant but varied the diversity and composition of plant assemblies at the greenhouse and plot scale. Since then increasingly sophisticated designs were devised incorporating also information on functional traits of component plant species in order to pinpoint the underlying mechanisms linking biodiversity to ecosystem functioning. More recently, the new field of functional biogeography has emerged trying to translate the above questions to regional and continental scales. It faces two main challenges, the high spatial heterogeneity of the abiotic template and the vast number of species (and their traits) and species combinations encountered at larger scales. Dealing with this requires `big data` sets of environmental variation, community composition and plant traits. Such datasets have only become available since a few years. In my talk I will highlight some important milestones of functional biogeography as well as recent work of my lab exploring the interaction between climate, species and functional diversity/identity and productivity across the North American and European temperate and boreal forests. Implications for global vegetation models are discussed.

5.4 TALK 2 – SYMPOSIA 3 – 11:00–11:20

Tracing plant-plant interactions on the spatial scale using community 15N isoscapes

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Plant-plant interactions are key processes shaping plant communities. However, the complex spatial dependencies of these processes are seldom addressed. Here we show that spatially resolved information on stable nitrogen isotopic signatures, i.e. 15N isoscapes, can be used to accurately trace N input following plant invasion by a nitrogen fixing species, *Acacia longifolia*, to a nutrient poor Portuguese dune system. Isotopic signatures of N differed strongly between the native system (δ15N ≈ -10‰) and the atmospherically derived N in *A. longifolia* phyllodes (δ15N ≈ 0‰). Thus, sources of N for native plants could be readily distinguished and N provided by *A. longifolia* could actually be mapped in the native system, using foliage of a non-fixing species, *Corema album*, as a biomarker. Geostatistical methods furthermore allowed quantifying the area affected by invasion, which was at least 3.5 times larger than the area occupied by the invader (Rascher et al. 2012). Thus, the isoscapes approach proved a valuable means for assessing the spatial dimension of functional changes associated with plant invasions. Moreover, considering the feasibility and applicability of this approach, it may provide a promising tool to identify, quantify and monitor different types of functional plant-plant interactions within communities at a spatially explicit scale.

Rascher et al. 2012, Ecol. Lett. 15: 484-491

5.4 TALK 3 – SYMPOSIA 3 – 11:20–11:40

How did the photorespiration change in the context of C4 evolution?

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The world's most prominent enzyme ribulose bisphosphate carboxylase/oxygenase (RuBisCO) cannot just fixate CO2, it is also able to fixate O2. Result of the oxygenation reaction is the toxic molecule 2-phosphoglycolate. This molecule has to be recycled to 3-phosphoglycerate in a costly process called photorespiration. To avoid photorespiration the C4 photosynthesis evolved. This photosynthesis type is based on the suppression of RuBisCOs oxygenation reaction via an enrichment of CO2 around RuBisCO. Therefore C4 plants separate the CO2 primary fixation in the Mesophyll tissue from its final fixation in the Bundle Sheath tissue of the leaf. Along with RuBisCO most of the photorespiratory enzymes are restricted to the Bundle Sheath tissue in fully-fledged C4 plants like *Zea mays*. This work points to the question how the photorespiratory gene expression changed in the context of C4 evolution. To answer that question we use the genus *Flaveria* that has a more recent C4 origin than *Z. mays* and contains C4, C3 as well as C3/ C4 intermediate plants and is therefore useful for evolutionary studies. By using plants with this different photosynthesis types we try to firstly, analyze the changes in the localization of different photorespiratory mRNAs and secondly the changes in photorespiratory promoter activity on the example of photorespirations entry enzyme 2-phospho glycolatephosphatase.

5.4 TALK 4 – SYMPOSIA 3 – 11:40–12:00

Homospermidine synthases in various Poaceae species

Anne-Maria Wesseling[1], Dietrich Ober[1]

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Pyrrolizidine alkaloids (PAs) are poisonous products of plant secondary metabolism found in angiosperms. These chemical compounds have evolved as a defense mechanism against herbivory. The first pathway specific enzyme of PA-biosynthesis is homospermidine synthase (HSS). This enzyme recruits its substrates from primary metabolism to synthesize the PA-precursor molecule homospermidine. Phylogenetic analyses revealed that HSS in different PA-producing plant species evolved from a copy of the deoxyhypusine synthase gene following several independent gene duplication events within the angiosperms. Although PAs had been studied for decades the occurrence of PAs in representatives of the grass family (Poaceae) was only discovered relatively recently. Therefore, we investigate and characterize PA biosynthesis in grasses by identifying HSS and DHS genes for phylogenetic analyses amongst others. After examination of several grass species we have discovered not one but two more independent gene duplication events which gave rise to two separate lineages of functional HSS genes in the two sister clades of Poaceae. This serves us as a basis for further analysis on the evolution of HSS and on PA biosynthesis in grasses generally.

5.5 TALK 1 – SYMPOSIA 7 – 13:30–14:00

Exploring the Genomes of Mycorrhizal Fungi to Understand the Evolution of Symbiosis

Francis Martin[1]

[1]INRA, Lab of Excellence ARBRE, Champenoux, France

Genomics has introduced an important new dimension into mycorrhizal research by establishing data to serve as a new and fundamental resource for genetics and molecular biology of the symbiosis formation. With the current genomic view of ectomycorrhizal (EM) fungi that we have, a possible scenario suggests that (1) irreversible losses of lignocellulose decomposition pathways play a key role in the evolutionary stability of the ectomycorrhizal mutualisms and (2) that each major EM fungal clade has subsequently and independently designed symbiotic molecular toolboxes each time the mycorrhizal lifestyle has arisen in the tree of life. The Mycorrhizal Genomics Initiative targets a set of 30 fungal mycorrhizal species selected for their ability to establish different types of mycorrhizal symbioses. I will discuss how the comparative analysis of these mycorrhizal genomes has, and will continue, to shed light on the evolution of mycorrhizal symbioses and how to harness environmental genomics of mycorrhizal systems to get a better understanding of carbon sequestration and biogeochemical cycles in forest ecosystems. Acknowledgments. The Mycorrhizal Genome Initiative is supported by the U.S. Department of Energy Joint Genome Institute. I would like to acknowledge the invaluable support of the MycorrhizalGenomics Initiative consortium.

5.5 TALK 2 – SYMPOSIA 7 – 14:00–14:20

Simultaneous application of heat, drought and virus to *Arabidopsis thaliana* plants reveals significant shifts in signaling networks

Uwe Sonnewald[1], Christian Prasch[1]

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Results of independently calculated climate models predict increased incidences of combined drought and heat stress which will considerably influence plant-pathogen interactions. To shed some light on molecular plant responses to multiple stress factors, a multi-factorial test system was developed, allowing simultaneous application of heat, drought and virus stress. Comparative transcriptome and metabolome analysis of single, double and triple stress responses revealed that gene expression under multi-factorial stress is not predictable from single stress treatments. Hierarchical cluster and principal component analysis identified heat as the major stress factor clearly separating heat-stressed from non-heat stressed plants. We identified 11 genes differentially regulated in all stress combinations as well as 23 genes specifically-regulated under triple stress. Furthermore, we showed that virus treated plants displayed enhanced expression of defense genes, which was abolished in plants additionally subjected to heat and drought stress. Triple stress also reduced expression of genes involved in the R-mediated disease response and increased the cytoplasmic protein response which was not seen under single stress conditions. The functional relevance of differentially expressed genes is analyzed in T-DNA knockout lines and different ecotypes. Results obtained will be discussed.

5.5 TALK 3 – SYMPOSIA 7 – 14:20–14:40

Environmental variability promotes plant invasion

Madalin Parepa[1], Markus Fischer[1], Oliver Bossdorf[1]

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Global environmental change not only entails changes in mean environmental conditions but also in their variability. Changes in climate variability are often associated with altered disturbance regimes and temporal patterns of resource availability.

Question: Such resource fluctuations may be a key determinant of habitat invasibility. However, although this hypothesis has been proposed over a decade ago, clear-cut experimental tests of its predictions are still rare. Methods: To test the potential effects of increased environmental variability on plant invasions, we conducted an ecological experiment in which one of the world's most invasive plants, Japanese knotweed, invaded experimental communities of native plants while changing either the amount or the variability of nutrients supplied. Results: Japanese knotweed success, is two- to fourfold increased in experimental plant communities if extra nutrients are not supplied uniformly, but in a single large pulse, or in multiple pulses of different magnitudes. Conclusion: The superior ability to take advantage of variable environments may be a key mechanism of knotweed dominance, and possibly many other plant invaders. Our study demonstrates that increased nutrient variability can promote plant invasion, and that changes in environmental variability may interact with other global change processes and thereby substantially accelerate ecological change.

5.5 TALK 4 – SYMPOSIA 7 – 14:40–15:00

Hygroscopic leaf surface particles reduce the drought tolerance of Scots pine by deliquescence, stomatal penetration and the establishment of wick-like structures

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Aerosols have always been part of the natural environment of plants, and plant leaves are a major sink for aerosol deposition[1]. Existing ecophysiological concepts tacitly assume clean leaf surfaces, but particles do affect plant water relations[2]. Many aerosols are hygroscopic and become deliquescent within the humid boundary layer of transpiring leaves. The surface tension of these highly concentrated solutions changes according to the Hofmeister series[3]. Needles of Scots pine (*Pinus sylvestris*) were sprayed with different salt solutions. Salt residues were observed within an environmental scanning electron microscope (ESEM) under varying humidity. Minimum epidermal conductance (gmin), an indicative parameter of drought tolerance, was determined. Concentrated salt solutions developed and chaotropic salts infiltrated into epistomatal chambers. The gmin increased for all salts, with strongest increase for a mixture of sea salt with surfactant. The 'hydraulic activation of stomata' is caused by the penetration of deliquescent salts into stomata, extending the liquid water phase from the apoplast to the leaf surface[4]. Increased gmin indicates uncontrollable water loss by these thin wick-like structures.

[1]Burkhardt, Ecol. Monographs, 2010

[2]Pariyar et al., Env. Exp. Bot. 2013

[3]Burkhardt et al., New Phyt., 2012

[4]Burkhardt & Pariyar, Env. Poll., 2013.

5.6 TALK 1 – SYMPOSIA 5 – 16:00–16:30

Problems, advances and next challenges in field studies of soil fungi diversity

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[1]Helmholtz Centre for Envioronmental Research (UFZ), Soil Ecology, Halle (Saale), Germany

Fungi comprise more than 1.5 Mio species that all are heterotroph and live as saprotrophs, symbionts or parasites. This range of strategies and wide physiological capacities make them to key players within soil habitats. However, soil fungi are mostly cryptic and not cultivable, so that their biodiversity is difficult to tackle. In addition because fungi are well-adapted to colonize the myriad of microhabitats of soils, it is especially challenging to relate time space variations in their community to environmental factors. The presentation will review the rapid development of molecular techniques used in soil fungal ecology to target neutral genome markers or regions encoding proteins. One particular challenge is to separate the active from the dormant communities within soils. This was attempted by targeting RNA soil extracts instead of DNA, which however, also introduces biases related to knowledge gaps in fungal genomics. The actual approaches use next generation techniques that enable high through put and complex correlation analyses. Examples will be given from large interdisciplinary projects such as the German Biodiversity Exploratories and BEF China. Apart disentangling diversity and community composition in relation to field factors, these approaches enable to depict interaction networks and co-occurrence patterns, which appear to be promising ecological descriptors.

5.6 TALK 2 – SYMPOSIA 5 – 16:30–17:00

Communities of arbuscular mycorrhizal fungi in tropical mountains of South Ecuador

Ingeborg Haug[1], Jutta Bloschies[1], Juan Pablo Suárez[2]

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Arbuscular mycorrhizal fungal diversity and composition is still relatively unknown in many ecosystems and especially in the tropical mountains. We investigated the communities of arbuscular mycorrhizal fungi of trees and shrubs in the evergreen premontane tropical forest (1000 m o.s.l.), in the tropical montane rain forest (2000 m o.s.l.) and from high altitude subpáramo (2900 - 3400 m o.s.l.) in the Podocarpus National Parc, South Ecuador. The arbuscular mycorrhizal fungi were analysed with molecular methods sequencing part of the 18S rDNA. The sequences were classified in Operational Taxonomic Units (OTUs) and analysed with phylogenetic methods. Our studies revealed an astonishing richness of arbuscular mycorrhizal fungi. Members of Glomerales dominated at 1000 m and 2000 m; at 3000 m members of Acaulosporaceae occurred in higher numbers. We found preferences for the multi-sampled plant species *Cedrela montana* Moritz ex Turcz. and *Tabebuia chrysantha* (Jacq.) G.Nicholson among AMF, but did not identify plant species with specific OTUs. We also found no indication for specificity of AMF. Networks of AMF and plants show significant nested structures in our study: Plant species with few AMFs in their roots associate with those AMFs recorded in many plant species. The diversity of plants is closely linked to diversity of mycorrhizal fungi maintaining rare species by mutualistic interactions with common partners.

5.6 TALK 3 – SYMPOSIA 5 – 17:00–17:30

Carbon allocation trade-off between arbuscular mycorrhizal fungi and roots reveals contrasting foraging strategies in pioneer plant species on sand

Ingo Höpfner[1], Martina Friede[1], Stephan Unger[1], Wolfram Beyschlag[1]

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Nutrient-poor, open sand ecosystems are of interest regarding plant nutrition and its relation to successional progress. In these systems efficient nutrient acquisition mechanisms are crucial for species success. In this regard most studies emphasize the advantages of extensive fine root systems. However, some successful pioneer species develop only coarse root systems, raising the question for alternative nutrient acquisition strategies. We hypothesize (i) that these plants allocate high proportions of C to arbuscular mycorrhizal fungi (AMF) to substitute for root surface and (ii) that nutrient depletion via AMF is equally efficient as via extensive fine root systems. To examine these hypotheses we performed a controlled experiment with five pioneer plant species: three forbs (*Plantago lanceolata*, *Hieracium pilosella*, *Hypochoeris radicata*) and two grasses (*Festuca psammophila*, *Corynephorus canescens*). In contrast to the grasses all forbs were highly dependent on mycorrhiza. Accordingly, data on root and hyphal growth confirmed contrasting strategies with predominant C-investment into AMF or roots in forbs and grasses, respectively. Soil P and N depletion rates proofed AMF as an equally efficient foraging strategy as fine root allocation. Our study emphasizes the importance of fungal parameters to clarify the relationship between species-specific traits and succession.

Session 5.7 – Evolutionary Relationships of Primary and Secondary Metabolism

Wednesday 10/02 – 15:30-17:00 – Chair: Birgit Draeger

5.7 TALK 1 – SYMPOSIA 8 – 15:30–16:00

A key alkaloid enzyme, putrescine *N*-methyltransferase, as example for evolution from a primary metabolic enzyme

Anne Junker[1], Juliane Fischer[2], Yvonne Sichhart[1], Wolfgang Brandt[2], **Birgit Draeger**[1]

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Plants display enormous chemical diversity. The evolution of the biosynthetic machinery (e.g. enzymes, transcriptions factors) is believed to result from gene duplication and specialisation of one gene copy. This concept, however, leaves many questions open: How are both gene copies kept, if most mutations lead to rapid non-functionalisation and pseudogene formation? How is a biosynthetic sequence with many metabolites realised, if only the end product provides selection advantages? Examples for detailed models of secondary product evolution will be discussed in this symposium. One example are putrescine *N*-methyltransferases (PMTs) in the biosynthesis of nicotine and tropane alkaloids. PMTs transfer a methyl group onto putrescine from *S*-adenosyl-l-methionine (SAM) as coenzyme. PMT proteins have presumably evolved from spermidine synthases (SPDSs), ubiquitous enzymes of polyamine metabolism. SPDSs use decarboxylated SAM as coenzyme. How many mutations does this neofunctionalization need? Mutagenesis of *Datura stramonium* SPDS1 and PMT generated PMT activity in SPDS1 after few amino acid exchanges. *Arabidopsis thaliana* SPDS1 after equivalent mutations yielded enzymes with both, PMT and SPDS activities. The rapid generation of PMT activity in SPDS and the wide-spread occurrence of putative products of *N*-methylputrescine suggest that PMT activity is present frequently in the plant kingdom.

Front. Plant Sci. 2013, doi: 10.3389/fpls.2013.00260

5.7 TALK 2 – SYMPOSIA 8 – 16:00–16:20

Secondary metabolites in early land plants: Rosmarinic acid production of the hornwort *Anthoceros agrestis*

Soheil Pezeshki[1], Maike Petersen[1]

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Bryophytes (liverworts, mosses and hornworts) are the earliest known land plants. Among these, the hornworts (Anthocerotophyta) are regarded as a sister clade to the tracheophytes. Although hornworts do not contain lignin, they have a broad phenolic metabolism. Suspension cultures of *Anthoceros agrestis* accumulating up to 6% of the dry weight rosmarinic acid (RA) are used to investigate the enzymes of the rosmarinic acid biosynthesis. The two entrypoint enzymes of the production of rosmarinic acid, phenylalanine ammonialyase (PAL) and tyrosine aminotransferase (TAT) show high specific activities during the first 7 days of a two week culture period. While PAL displays an apparent Km value of approx. 45 µM for L-phenylalanine, TAT has an apparent Km of approx. 7 mM for tyrosine and 9 mM for 2-oxoglutarate. Techniques of molecular biology are used to obtain sequence information to heterologously synthesise PAL, TAT and a hydroxycinnamoyltransferase (HCT) proteins for further kinetic experiments and to get information about the evolutionary relationships between RA biosynthesis in Lamiaceae and Anthcerotaceae. The corresponding amino acid sequences reveal high similarity of *Anthoceros* PAL to PAL from Lamiaceae but substantial differences of the HCT from *Anthoceros* to those in Lamiaceae were observed.

5.7 TALK 3 – SYMPOSIA 8 – 16:20–16:40

Diversity of ‚cineole cassette' monoterpene cyclases in the genus *Nicotiana*

Birgit Piechulla[1], Anne Brosemann[1], Anke Fähnrich[1], Madeleine Neumann[1]

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Flowers of the genus *Nicotiana* emit a characteristic set of so called ‚cineole cassette' monoterpenes comprizing 1,8-cineole, myrcene, sabinene, limonene, alpha- and beta-pinene, alpha-terpineol. Several monoterpene synthases of various *Nicotiana* species were isolated and characterized as terpineol (TER) and cineole (CIN) synthases. The cyclization reaction of terpineol to 1,8-cineole is an addition reaction (hydroxyl group of the precursor alpha-terpineol reacts with the double bond to introduce the second intramolecular cycle). Apparently the efficacy of this cyclization reaction is different in the enzymes of the *Nicotiana* species. A site-directed mutagenesis approach is performed to identify amino acids with impact on the cyclization reaction. In addition, our investigations address the question about the evolution of the monoterpene synthases of *Nicotiana* species. Species of section *Alatae* emit 'cineole cassette' monoterpenes, while species of the sister section *Suaveolentes* do not, although it was hypothesized that one parent of section *Suaveolentes* is a member of the present section *Alatae*. Thus, we started to search for CIN or TER progenitor enzymes. This approach will indicate gain or loss of functions during evolution and identify the original terpene synthase before *Alatae* (South America) and *Suaveolentes* (Australia) divierged during glacial age.

5.7 TALK 4 – SYMPOSIA 8 – 16:40–17:00

Function and interaction of cytochrome P450 enzymes in the biosynthesis of camalexin

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Camalexin is the characteristic phytoalexin of Arabidopsis and important for defense against a number of fungal pathogens. Its biosynthetic pathway from tryptophan and glutathione involves multiple steps catalyzed by partially redundant cytochrome P450 enzymes of the 79B and the highly expanded 71A and 71B subfamilies. Here, CYP71A12, CYP71A13 and CYP71A18 similarly convert the intermediate indole-3-acetaldoxime (IAOx) to indole-3-acetonitrile (IAN) in vitro, but strongly differ in the extent indole-3-aldehyde is formed as a side product. Specific for camalexin biosynthesis, an exceptional bifunctional P450, CYP71B15 (PAD3), catalyzes cyanide release from Cys(IAN) under formation of a thiazoline ring as well as the subsequent decarboxylation yielding camalexin. In response to biotic stress, *CYP71B15p*:CYP71B15-GFP is highly expressed specifically in cells in contact with pathogens. We screened for interaction partners of CYP71B15-GFP by a series of untargeted Co-IP experiments and found cytochrome P450s being highly enriched, including known enzymes of glucosinolate and camalexin biosynthesis, such as CYP71A13. This indicates that biosynthetic complexes are formed.

6.1 TALK 1 – SYMPOSIA 2 – 16:00–16:30

Design your genome! How to create a plant with the chromosome composition and the cytoplasmic genome of your choice.

Erik Wijnker[1], Laurens Deurhof[2], Jose van de Belt[2], Bas de Snoo[3], Frank Becker[2], Ravi Maruhachalam[4], Sara Movahedi[3], Remco Ursum[3], Cilia Lelivelt[3], kees van Dun[3], Hans de jong[2], Joost Keurentjes[2], Rob Dirks[3]

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In 2012 we showed the feasibility of reverse breeding, an anticipated plant breeding technique in the model plant Arabidopsis. It was shown that using reverse breeding it is possible to -relatively easy- chromosome substitution lines in Arabidopsis. In its essence, the technique allows one to exchange one, several or all chromosomes between two different Arabidopsis accessions. The technique was developed after the discovery of a method for the production of (doubled) haploids in Arabidopsis: the CENH3-tailswap haploid inducer (Ravi and Chan, 2010; doi: 10.1038/nature08842). We have been exploring the use of this mutant for further uses in recent years. Interestingly, this haploid inducer also allows for the generation of cybrids: plants that carry the nuclear genome of one plant and the cytoplasm (chloroplasts/mitochondria) of another. Taken together, reverse breeding and the possibility of generating cybrids lead to an interesting "toolbox" that allows the generation of Arabidopsis "designer genomes". It is possible to control both the nuclear chromosome composition of plants and combine such designer nuclear genomes with any cytoplasm of choice. This allows for the development of new approaches in the study of -for example- epistatic interactions en heterosis.

6.1 TALK 2 – SYMPOSIA 2 – 16:30–16:50

Hybrid Performance Prediction in Winter Wheat Based on Genomic and Metabolomic Data — Metabolite Profiling —

Andrea Matros[1], Jochen Christoph Reif[2], Yusheng Zhao[2], Hans-Peter Mock[1]

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The project aims at the development of accurate and robust hybrid prediction methods using a vast genotypic and phenotypic dataset with metabolite profiles of wheat lines and their crosses. Here a mapping population comprising 1604 hybrids and their 135 parental winter wheat lines is used to predict their combining abilities for a number of grain yield- and quality-related traits by means of their genetic fingerprints using the Infinium iSelect 9K wheat array and their metabolite profiles as determined by gas chromatography (GC) combined to mass spectrometry (MS). We will present our approach for the untargeted metabolite profiling of selected lines of the mapping population. Field trials have been conducted for all the aforementioned lines. For metabolite profiling ten flag leaves were harvested from three individual environments at three different developmental stages for selected lines, each. Untargeted GC-MS profiling was performed to cover a wide range of substances. Extraction protocols and GC-MS analysis techniques were optimized for wheat flag leaf samples. N-alkane standard was used to adjust retention time shifts, and a mixed reference sample as well as labelled glucose was used to adjust ion intensity shifts during measurements. Our workflow for data processing, feature extraction, relative quantification, and statistics will be presented. Initial results from biometrical models for prediction of hybrid performance with metabolites as predictor variables will be discussed.

6.1 TALK 3 – SYMPOSIA 2 – 16:50–17:10

Enhancing the root system of barley (*Hordeum vulgare* L.) using CKX technology

Eswar Ramireddy[1], Heike Gnad[2], Jens Weyen[3], **Thomas Schmülling**[1]

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Roots are relevant to plants for sensing and transporting nutrients and water from the ground. Therefore, root system architecture (RSA) has become a major target for attempts to increase tolerance to abiotic stresses such as nutrient deficiency, drought and salinity. We have investigated the role of cytokinin in regulating RSA in barley and developed strategies to achieve root enhancement. Cytokinin is a negative regulator of primary root growth and lateral root formation. Consistently, lowering the cytokinin content by ectopic expression of cytokinin-degrading *CKX* genes in roots causes increased primary root elongation and enhanced lateral root formation in dicots. Plants with an enhanced root system were more tolerant to drought and nutrient deficiency (Werner et al., Plant Cell 22: 3905-3920, 2010). We investigated in barley whether a lower cytokinin status of roots has similar consequences for RSA in monocot plants. We first identified four root-specific promoters in rice and fused these to *CKX* genes of *Arabidopsis*. Transgenic barley lines containing eight different promoter::*CKX* combinations were obtained. Plants with a predominant transgene expression in roots were identified and shown to form an increased root system in a hydroponic test system. The performance of plants with an enhanced root system under different environmental conditions will be investigated.

6.1 TALK 4 – SYMPOSIA 2 – 17:10–17:30

Designer endonuclease-mediated gene targeting in barley

Maia Gurushidze[1], **Goetz Hensel**[1], Stefan Hiekel[1], Sindy Schedel[1], Vladimir Valkov[1], Jochen Kumlehn[1]

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Transcription activator-like (TAL) effectors from pathogenic bacteria of the genus *Xanthomonas* are excreted into infected plant cells and bind to specific DNA motifs to manipulate host gene expression. The DNA-binding domain of TAL effectors consists of basically four types of repeats, each having specific affinity to one of the four nucleotides. This principle allows us to design sequences to code for DNA-binding domains with specificity to genomic target sequences of choice. In TAL effector nucleases (TALENs), such designer DNA-binding domain is coupled to a *Fok*I nuclease which facilitates the creation of double strand breaks (DSBs) at a user-defined genomic position. DSBs are then processed by the cell's DNA repair machinery, which is error-prone to some extent and thus causes mutated target sites. To establish gene targeting technology in a cereal crop, a *gfp* specific TALEN pair was designed and used for gene transfer in barley embryogenic pollen cultures made from *gfp* lines. In this setup, a deleterious mutation of just one *gfp* allele is effective due to the haploid nature of the pollen-derived target cells, which could be converted into homozygous plant upon genome duplication. Screening for loss of fluorescence and sequencing the *gfp* gene of TALEN transgenics resulted in the detection of *gfp* knock-out mutants that were then analysed for genetic homogeneity and generative transmission.

6.2 TALK 1 – SYMPOSIA 8 – 15:30–16:00

Glyco-engineering for plant-based biopharmaceutical production

Eva Decker[1]

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As protein therapeutics represent a strongly growing area in the pharmaceutical industry pharmaceutical proteins become especially interesting within the field of molecular farming, i.e. the large-scale production of recombinant proteins. Plant-based biopharmaceutical production is gaining acceptance as an alternative for currently used mammalian cell systems. Different plant systems have been established which are suitable for standardisation and precise control of cultivation conditions, thus meeting the criteria for pharmaceutical production. As the majority of biopharmaceuticals comprise glycoproteins, plant-specific glycosylation has to be taken into account and possibly engineered to human-like or identical patterns. Gene targeting as well as knockdown strategies were employed to diminish the activity of plant-specific glycosyltransferases and mammalian genes were introduced into plant hosts to reconstruct the biosynthesis pathways for mammalian N- and O-glycosylation.

References:

EL Decker & R Reski: Glycoprotein production in moss bioreactors. Plant Cell Rep 2012, 31: 453-460.

D Bosch et al: N-Glycosylation of plant-produced recombinant proteins. Curr Pharm Des 2013, 19: epub ahead of print.

6.2 TALK 2 – SYMPOSIA 8 – 16:00–16:20

Plant Seeds as a Productionsystem - comparative analysis of Plant made Pharmaceuticals (PMP) and Industrials (PMI) produced in different species

Christoph Unger[1], Susanne Baars[1], Jana Huckauf[1], Heike Mikschofsky[1], Daniel Ponndorf[1], Nan Qu[1], Uwe Kahmann[2], Inge Broer[1]

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Biodiversity in crop rotation might be increased by the additive production of high value compounds likePlant made Pharmaceuticals (PMP) and Industrials (PMI)in crops that are currently of more ecological the economic interest. As production platform plant seeds have several advantages such as high protein yield and stable storage of target proteins. The biopolymer Cyanophycin and a vaccine against rabbit haemorrhagic disease virus (RHDV) CTB::VP60 provide the model substances for our project. Arabidopis, Tobacco and pea serve as production plattforms. Two different seed specific promoters were used in comparison and fused either to the coding region for the Cyanophycin synthetase and of the CTB::VP60 gene. High amounts of the transgene encoded proteins could be achieved.

6.2 TALK 3 – SYMPOSIA 8 – 16:20–16:40

Protein quality control - from protein recognition and degradation to conditional protein expression

Frederik Faden[1,2], Christin Naumann[1,2], Maria Bongartz[1,2], Pavel Reichman[1,2], Carolin Mai[1,2], Anne Kind[1,2], Florian Sperling[1,2], Michaela Reissland[1,2], **Nico Dissmeyer**[2]

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The ON/OFF status of *functional* proteins within a cell's proteome must be precisely controlled to ensure its proper life by checkpoint-like protein quality control (PQC) mechanisms. PQC also kicks in if proteins are "used up" after their action and thus need to be removed from the cell. In plants, PQC is important for breakdown of storage reserves in seeds, germination, leaf and shoot development, flower induction, cell division, and possibly plant-pathogen interaction. Functional plant proteins as one of the premier storage units for energy are hallmarks of plant development and their environmental stress tolerance, but also in the light of producing plant-made pharmaceuticals. We functionally analyze novel enzymatic components and substrates with special emphasis on the N-end rule (NERP). In plants, NERP is poorly understood. We have developed an *in vivo* transgenic protein stability reporter system. Our laboratory work is mainly focused on studies of enzymatic NERP components (E3 Ubiquitin ligases, arginyl-transferases, and amidases), their substrate proteins as well as on the use of protein expression "on demand" as biotechnological applications in plants. Therefore, we are establishing conditional stabilization/destabilization assays of diverse functional classes of proteins such as enzymes, transcription factors, storage and reserve proteins but also toxic and large proteins with difficult folds which might be used in molecular farming.

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6.2 TALK 4 – SYMPOSIA 8 – 16:40–17:00

New Green Chemistry: methane production by the action of phototrophic microalgae without biomass formation

Christian Wilhelm[1], Anja Günther[1], Theresa Quaas[1], Torsten Jakob[1], Reimund Goss[1], Swetlana König[1], Daniel Spindler[1], Susann Reinert[1], Norbert Räbiger[2], Saskia John[2], Susanne Heithoff[2], Mark Fresewinkel[3], Clemens Posten[3]

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Detailed analysis of the rate limiting step in the conversion of light into biomass has shown that the major energy losses are due to the metabolic conversion of the photosynthates into biomass. These losses can not be drastically reduced by „metabolic engineering", because the limitations are of physical nature. Life cycle studies revealed evidence that the emission of green house gases produced by cultivation, harvest and refinement are still too high for sustainable biofuel generation. The reasons for the energetically inefficient microalgal production process is the unfavourable ratio of energy input for the mass transport to the energy content of new biomass. These physiological and technical constraints of biomass production have been circumvented in the concept of „New Green Chemistry". Here, the algae are handled as an active biofilm reducing the energy input for mass transport by several orders of magnitude. Second, photosynthates produced by the calvin cylcle are not used for growth. Instead the carboxilation/oxygenation ratio is adjusted to 2:1 to produce glycolate only. The excreted glycolate is transferred to an anaerobic compartment where it is converted to methane by the action of methanogenic microbes. The presentation gives the state of the art of this approach and shows the future potential of this new biotechnological design.

Session 7.1 – Systemic Approaches I: Genomics and Next Generation Sequencing

Monday 09/30 – 16:00-17:30 –
Chair: Korbinian Schneeberger

7.1 TALK 1 – SYMPOSIA 2 – 16:00–16:30

Phylogenetic shadowing guided by the cycling transcriptomes of two distantly related Brassicaceae species reveals the atlas of cis-regulatory elements of daytime-specific and daytime-unspecific pathways

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Recent genome comparisons of a growing number of Brassicaceae genome assemblies revealed the extent of conserved non-coding sequences (CNS). Despite these evolutionary constraints, little is known about their function. We assembled the genome of *Arabis alpina*, which diverged ~30 million years ago from *A. thaliana* and sequenced the transcriptomes of both species at 12 time points of two consecutive days. Over ~4,000 orthologous genes showed daytime-dependent expression. Comprehensively analyzing CNS for cis-regulatory elements revealed 50 putatively daytime specifying motifs, including all those shown to be involved in diurnal expression so far. Moreover, absence or presence of many elements has significant impact on the degree of conservation in gene expression, evidencing their role in gene regulation. On average diurnal genes are delayed by two hours in *A. alpina* as compared to *A. thaliana*. The plant circadian clock can be divided into three intervening loops, but intriguingly this shift is only present in the morning and evening loops, but not in the core loop. GO enrichment and pathway analyses revealed striking differences between pathways conserved for their daytime and those being shifted. Conserved pathways include photosynthesis and primary metabolite biosynthesis, whereas shifted pathways are enriched for secondary metabolites biosynthesis.

7.1 TALK 2 – SYMPOSIA 2 – 16:30–16:50

Complementation contributes to transcriptome complexity in maize (*Zea mays* L.) hybrids relative to their inbred parents

Anja Paschold[1], Yi Jia[2], Caroline Marcon[1], Steve Lund[3], Nick B. Larson[3], Cheng-Ting Yeh[2], Stephan Ossowski[4], Christa Lanz[4], Dan Nettleton[3], Patrick S. Schnable[2], Frank Hochholdinger[1]

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F1-hybrids are more vigorous than their homozygous, genetically distinct parents, a phenomenon known as heterosis. The molecular mechanisms underlying this phenomenon are only poorly understood. In the past it was demonstrated that already the young root system shows heterosis. In the present study the transcriptomes of the reciprocal maize (*Zea mays* L.) hybrids B73xMo17 and Mo17xB73 and their parental inbred lines B73 and Mo17 were surveyed in primary roots early in the developmental manifestation of heterosis. The application of novel and robust statistical approaches and a suitable experimental design established that 34,233 (i.e., 86%) of all high-confidence maize genes were expressed in at least one genotype. Consistent with the dominance model (i.e., complementation) for heterosis 1,124 genes that were expressed in the hybrids were expressed in only one of the two parents (=**s**ingle **p**arent **e**xpression). For 65 genes it was shown that SPE was a consequence of complementation of genomic presence/absence variation. The remaining 1,065 genes were identified as true gene expression variations between the parental inbred lines which were complemented in the hybrids. As a consequence, both hybrids expressed more genes than did either parental inbred. The ability to compensate for alleles not expressed in one of the two parents might be a mechanism contributing to hybrid vigor.

7.1 TALK 3 – SYMPOSIA 2 – 16:50–17:10

Comparative transcriptome atlases in Cleome

Canan Külahoglu[1], Alisandra Kaye Denton[1], C Robin Buell[2], Andrea Bräutigam[1], Andreas PM Weber[1]

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C4 photosynthesis is an adaptive trait that allows plants to survive in hot and arid environments. The establishment of C4 photosynthesis requires several alterations in leaf anatomy, biochemistry and leaf development. The metabolic genes for C4 photosynthesis are known, but the underlying regulatory network is poorly understood. We hypothesize that the changes in C4/C3 related gene expression are controlled by a subset of transcriptional programs, which are essential for C4 photosynthesis establishment and maintenance. We created transcriptome atlases for a C4 species, *Cleome gynandra*, and a closely related C3 species, *Tarenaya hassleriana* (6 leaf developmental categories, 3 seedling and 3 seed developmental stages, root, stem and floral tissues) by RNA-seq and supported the data by microscopy, metabolite profiling, enzyme assays and photosynthesis measurements. Co-expression analysis of leaf development resulted in 16 different modules in the C4 and 10 co-expression modules in the C3 plant. These modules shared distinct expression patterns, which in majority could be assigned to main developmental processes. A group of cell cycle genes exemplify the pattern of the data: most of the genes are expressed in a similar manner in both species, but a small number of genes exhibit different expression patterns. These genes are candidates for C4 related functions and are currently analyzed.

7.1 TALK 4 – SYMPOSIA 2 – 17:10–17:30

The unusual chloroplast genome sequence of the Synchromophyceae-like alga *Chrysopodocystis socialis* (Ochrophyta)

Maria Schmidt[1], Lasse Feldhahn[2], Mika Tarkka[2], Tesfaye Wubet[2], Christian Wilhelm[1]

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Chloroplast genomes play an important role in the understanding of organelle integration into the host cell, especially in the context of secondary or tertiary endosymbioses. Plastids of red algal origin can be found among haptophytes, cryptophytes, alveolates and heterokonts. Since previous attempts to sequence the plastome of the heterokont amoeboid alga *Synchroma grande* (Synchromophyceae, Ochrophyta) failed due to high amounts of DNA contaminants from co-cultivated bacteria, we attempted, for the first time, to enrich plastids of secondary red origin using fluorescence-activated cell sorting (FACS) prior to DNA extraction. To further increase DNA quantity whole genome amplification (WGA) was used. The first plastid genome of a Synchromophyceae-like alga, *Chrysopodocystis socialis*, is a circular molecule of ~126 kb with several unique features compared to the 15 heterokont algae plastomes sequenced so far. Two large inverted repeat regions of 16.6kb encase the smallest small single copy region (~ 2kb, 3 genes) reported. Also, a yet undescribed open reading frame was found, as well as a transporter usually only present in cryptophyte and rhodophyte plastomes. The presented chloroplast genome marks the foundation, upon which future sequencing attempts of the *Synchroma* species with chloroplast complexes can be based, e.g. using a long range PCR approach.

7.2 TALK 1 – SYMPOSIA 4 – 14:00–14:30

Spatial and temporal patterns of metabolites during barley seed development

Manuela Peukert[1], Andrea Matros[1], Winfriede Weschke[1], **Hans-Peter Mock**[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

Higher plants are composed of a multitude of tissues with specific functions, reflected by distinct profiles for transcripts, proteins and metabolites. Global analysis of metabolites and proteins has advanced tremendously within recent years, and this progress has been enabled by the rapid development of novel mass spectrometric instrumentation. In many of the current "omics"-studies, proteome or metabolite analysis is performed on whole organ or whole plant extracts. This approach leads to the loss of spatial information. Mass spectrometry imaging (MSI) techniques have opened a new avenue to obtain information on the spatial distribution of metabolites and of proteins. A range of different plant organs and tissues have been successfully analyzed by MSI, and patterns of various classes of metabolites from primary and secondary metabolism could be obtained. It can be envisaged that MSI approaches will substantially contribute to build spatially resolved biochemical networks of model plants and crops. We have applied this approach to elucidate spatially resolved metabolic networks related to barley grain development. We analyzed longitudinal and cross sections from developing barley grains (3, 7, 10 and 14 days after pollination). In the presentation we will address spatial resolution and sensitivity of the method. Identification of unknown compounds will also be discussed.

7.2 TALK 2 – SYMPOSIA 4 – 14:30–14:50

MultiPollenOmics reveals dynamic changes in pollen metabolism

Veronika Lang[1], Lena Fragner[2], Wolfram Weckwerth[2], Björn Usadel[3], Heidi Pertl-Obermeyer[4], **Gerhard Obermeyer**[1]

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Question: The major aim of pollen grains (PG) is the delivery of sperm cells for fertilization. A pollen tube (PT) is generated which grows rapidly towards the egg cells while facing changing conditions, e.g. different oxygen concentrations, on its way through the pistil and style tissue. To reach the egg cell first, the individual PTs have to adapt cellular processes and metabolism frequently to continue growth and to reach the egg cells first. Methods: Investigations of the lily pollen (Lilium longiflorum Thunb.) proteome, metabolome and transcriptome gave a comprehensive overview of metabolic pathways active during pollen germination and tube growth. Results: More than 80 protein/enzyme classes, >150 different metabolites and >5,000 partial enzyme sequences of components of the primary metabolism were determined and identified by LC-MS/MS, GC-MS and next generation sequencing. Conclusion: The time-dependent changes in metabolite abundances as well as the changes after inhibition of the mitochondrial electron transport chain (ETC) revealed a fast and dynamic adaption of the metabolic pathways in the range of minutes. The metabolic state of ungerminated PGs differed clearly from the metabolism during PT growth as indicated by principal component analysis. Inhibition of the ETC resulted in an immediate production of ethanol and a fast re-arrangement of metabolic pathways, e.g. activation of the GABA shunt.

7.2 TALK 3 – SYMPOSIA 4 – 14:50–15:10

Using Metabolite Fingerprinting to Elucidate Secondary Metabolism of Brassicaceae upon *Verticillium longisporum* Infection

Mareike Possienke[1], Farina Schill[1], Kirstin Feussner[1], Alexander Kaever[2], Christian Timpner[3], Manuel Landesfeind[2], Stefanie König[1], Susanna Braus-Stromeyer[3], Gerhard Braus[3], Andrea Polle[4], **Ivo Feussner**[1]

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Infection of oilseed rape (*Brassica napus*) with the hemibiotrophic fungus *Verticillium longisporum* leads to significant changes in the metabolome of the host plant. In order to identify specifically accumulating metabolites during infection, so-called infection markers, a metabolite fingerprinting analysis of oilseed rape was performed. For temporal and spatial resolution the metabolites were analysed from a time course of 5 to 35 days post infection and from different tissues (leaf, stem, hypocotyl and roots) and apoplastic fluids (xylem sap and apoplastic wash fluid) . Among the metabolites enriched in infected tissues were the phytoalexin cyclobrassinin and 22 related so far not described substances. Their structure was identified by fragmentation analysis. These mostly (malonyl-)glycosylated derivatives are supposed to accumulate during cyclobrassinin biosynthesis. Based on the data, a model for the biosynthesis of cyclobrassinin could be proposed in analogy to the model of camalexin synthesis in *Arabidopsis thaliana*. Additionally accumulation of 2-mercapto-indole-3-carboxylic acid derivatives was detected in infected plant tissues, which suggests a subsequent degradation of cyclobrassinin and indicates a balancing of the phytoalexin level *in planta*. Abiotic stress treatment of *B. napus* leaves mimicked many metabolite responses induced by the fungal pathogen showing the metabolic changes to be a general response to stress treatment of the plant.

7.2 TALK 4 – SYMPOSIA 4 – 15:10–15:30

Targeted and non-targeted Metabolite Profiling of Arabidopsis Root Exudates after Phosphate Starvation

Jörg Ziegler[1], Christoph Böttcher[1], Nadine Strehmel[1], Schmidt Stephan[1], Steffen Neumann[1], Dierk Scheel[1], Steffen Abel[1]

[1]Leibniz-Institute of Plant Biochemistry, Molecular Signal Processing, Halle, Germany

Roots secrete various ions and phytochemicals into the rhizosphere to increase the bioavailability of nutrients, of which phosphate (P*i*) is one of the most limiting one. Arabidopsis wild type plants exhibit strongly shortened primary root length upon P*i* deprivation. Mutants have been isolated, which are either hypersensitive (*pdr2, pdr3*) or insensitive (*lpr1/2*) to P*i* deficiency with respect to primary root growth. A sterile hydroponic system was developed which allows visualization of root phenotypes, sampling and processing of root exudates for non-targeted metabolite profiling by UPLC-ESI-qTOF-MS, and targeted metabolite profiling by HPLC-ESI-MS/MS. Arabidopsis plants can be separated according to their genotypes and +P*i* and - P*i* treatment based on non-targeted metabolite profiling of their root exudates. Additionally, all four genotypes showed little overlap in changes in root exudate composition in response to - P*i* treatment. All 20 standard amino acids were present in root exudates, but did not show differences in abundance between genotypes or treatments. Out of twelve plant hormones and plant hormone conjugates, seven (ABA, IAA, JA, JA-Ile, OPDA, SA, *trans*-Zeatin-9-riboside) could be shown to accumulate in root exudates, of which JA was predominant in samples derived from hypersensitive *pdr2* roots.

Session 7.3 – Systemic Approaches III: Proteomics and Phosphoproteomics

Wednesday 10/02 – 10:30-12:00 – Chair: Sacha Baginsky

7.3 TALK 1 – SYMPOSIA 6 – 10:30–11:00

Functional proteomics offers a new perspective on chloroplast biogenesis and the function of posttranslational modifications

Sacha Baginsky[1]

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Our research is focused on the characterization of basic chloroplast functions with functional proteomics. Two lines of research are currently prevalent: First, it is our goal to understand the assembly of the chloroplast proteome. To this end, we characterize the plastid protein import machinery, assess its dynamic subunit composition and aim at identifying new import components. At the functional level, we study the consequence of components lacking from the import machinery on the chloroplast proteome by quantitative proteomics, such as exemplified with the Toc159 mutant *ppi2* (Bischof et al., 2011, Plant Cell 23, 3911-28). Second, our research aims at deciphering the chloroplast phosphoproteome network entailing the identification of protein kinase substrates and the analysis of phosphorylation dynamics (e.g. Reiland et al., 2011, PNAS 108, 12955-60). The tools we use are comparative quantitative phosphoproteomics and the peptide chip ChloroPhos1.0 that allows the multiparallel analysis of *in vitro* phosphorylation activity on more than 900 substrate peptides (Schönberg and Baginsky, 2012, Front Plant Sci 3, 256).

7.3 TALK 2 – SYMPOSIA 6 – 11:00–11:20

Comprehensive cell-specific protein analysis in early and late pollen development - from diploid microsporocytes to pollen tube growth

Till Ischebeck[1,2], Luis Valledor[2], David Lyon[2], Stephanie Gingl[2], Mathias Nagler[2], Mónica Meijón[3], Volker Egelhofer[2], Wolfram Weckwerth[2]

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[2]University of Vienna, Molecular Systems Biology, Vienna, Austria
[3]Gregor-Mendel-Institute for Molecular Plant Biology, Vienna, Austria

Pollen development in angiosperms is one of the most important processes controlling plant reproduction and therefore productivity. Therefore pollen development is a major focus in applied studies and breeding approaches for improving plant productivity in a globally changing climate. To reveal a quantitative proteome map of pollen development we analyzed 8 stages of tobacco pollen development from diploid microsporocytes, meioses, tetrads, microspores, polarized microspores, bipolar pollen and desiccated pollen to pollen tubes. A protocol for the isolation of the early stages was established. Proteins were extracted and analyzed by a new Gel-LC-MS fractionation protocol. In total, 3817 protein groups were identified. Quantitative analysis was performed based on peptide count. Exceedingly stage-specific differential protein regulation was observed during the conversion from the sporophytic to the gametophytic proteome.

7.3 TALK 3 – SYMPOSIA 6 – 11:20–11:40

Proteomic analysis of the Cyanophora paradoxa muroplast provides clues on early events in carbon allocation in plants

Fabio Facchinelli[1], Mathias Pribil[2], Ulrike Oster[2], Nina Ebert[1], Debashish Bhattacharya[3], Dario Leister[2], Andreas P. M. Weber[1]

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Glaucophytes represent the first lineage of photosynthetic eukaryotes of primary endosymbiotic origin which diverged after plastid establishment. It is highly desirable to gain knowledge on the glaucophyte plastid composition in order to get insight into the evolutionary history of cyanobiont integration and plastid development. Here we provide the first proteomic analysis of the muroplast of *Cyanophora paradoxa*.Reconstruction of the ancient carbon fluxes from the cyanobiont to the host cytosol predicted that during endosymbiosis photosynthate was exported to the cytosol where it was polymerized from ADP-glucose into glycogen.The protein repertoire of the muroplast revealed novel paths for reduced carbon flow and export to the cytosol through a sugar phosphate transporter of chlamydial origin.Recently, Chlamydia-like pathogens turned out to be the second major source of foreign genes in Archaeplastida with a significant number of genes horizontally transferred. This hints at a significant role of these obligate intracellular pathogens during establishment of endosymbiosis, likely through facilitating the metabolic integration between the endosymbiont and the eukaryotic host. We here propose that an hexose phosphate transporter of chlamydial origin represented the first transporter responsible for exporting photosynthate out of the cyanobiont.

7.3 TALK 4 – SYMPOSIA 6 – 11:40–12:00

Exploring the role of lysine acetylation in the regulation of plant metabolism

Markus Hartl[1], Paul J. Boersema[2,3], Matthias Mann[2], Iris Finkemeier[1]

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Acetylation of the ε-amino group of lysine is a reversible post-translational modification recently discovered to occur on proteins outside the nucleus, in most sub-cellular locations in mammalian cells. Until recently, almost nothing was known about this modification in plants beyond the well-studied acetylation of histone proteins in the nucleus. We developed a protocol based on high resolution mass spectrometry for the identification and relative quantification of lysine-acetylated peptides in *Arabidopsis thaliana*. With this optimized method we mapped quantitative changes in the Arabidopsis leaf acetylome after particular genetic or biochemical manipulations. Our results indicate that lysine acetylation could be important in the regulation of key metabolic enzymes and protein complexes, including a large proportion of photosynthetic proteins. Central enzymes of the Calvin-cycle, as for example RuBisCO, are specifically and dynamically acetylated at various sites and deacetylation *in vitro* has a strong impact on enzyme activity. One of the main questions of our research is to elucidate whether lysine acetylation is important for *in vivo* enzyme functions, such as the regulation of photosynthesis and respiration, and includes the identification and characterization of organellar lysine acetyltransferases and deacetylases.

Session 7.4 – Quantitative Biology using Mathematical Modeling

Monday 09/30 – 14:00-15:30 – Chair: Christian Fleck

7.4 TALK 1 – SYMPOSIA 1 – 14:00–14:30

Dimerization determines specificity of Phytochrome B action

Christian Fleck[1]

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Phytochrome B (phyB) mediates the classical red/far-red reversible responses, whereas the light labile phyA is essential for the high-irradiance responses (HIR) in strong continuous far-red light. Intriguingly, the action spectrum for phyB mediated inhibition of hypocotyl elongation is more distinct from the phyA action spectrum than it could be expected from the calculated phyB action spectrum based on the photoequilibrium.These differences cannot be explained by the photophysical properties of phyA and phyB, because these are virtually identical. Although our previous integrative mathematical model of the action of phytochrome B (Rausenberger et al., 2010) could reliably predict the photon fluence rate response curves in red light, the model failed to reproduce the abrupt reduction in effectiveness at longer wavelengths. Considering the biochemical properties of phyB in vitro it is appears that dimerization is an integral part for the action of phyB, which was neglected in our previous model. In a combined approach between experiments and theory we were able to unravel the underlying principles leading to the strong drop of phyB function in wavelength longer than 690 nm. The discrimination of phyA and phyB action cannot be understood by the molecular properties of the phytochromes alone, but is evidently a systems property of the signaling network.

7.4 TALK 2 – SYMPOSIA 1 – 14:30–14:50

Strategies of mathematical modeling successfully link results from experimental high-throughput analysis of plant primary metabolism to complex biochemical regulation

Thomas Nägele[1], Hannes Doerfler[1], David Lyon[1], Xiaoliang Sun[1], Lena Fragner[1], Arnd G. Heyer[2], Wolfram Weckwerth[1]

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The comprehensive analysis of regulation strategies in plant primary metabolism is central to numerous studies focusing on plant-environment interaction. In this context, we present a strategy of mathematical modeling of primary metabolism in *Arabidopsis thaliana* to unravel steps of metabolic reprogramming during exposure to low temperature. We investigated reprogramming of central metabolic pathways at the cellular as well as the subcellular level after different periods of cold exposure. Our experimental high-throughput analysis covered pools of carbohydrates, carboxylic and amino acids as well as measurements of CO2 assimilation. Due to the multidimensional data matrix resulting from the analysis, intuitive inference of regulatory instances was precluded. Hence, we developed a strategy of mathematical modeling allowing the identification of steps of metabolic reprogramming. We derived a biochemical Jacobian matrix from experimental data, containing information about enzymatic reactions and intracellular transport processes of the underlying metabolic network. We identified hitherto unknown regulatory instances contributing significantly to the successful acclimation of *Arabidopsis thaliana* to low temperature. This proves the suitability of our approach to detect steps of metabolic reprogramming in a complex biochemical system.

7.4 TALK 3 – SYMPOSIA 1 – 14:50–15:10

Modelling SERK mediated BR signalling

Wilma van Esse[1], Simon van Mourik[2], Sacco de Vries[3]

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Brassinosteriods (BRs) are essential for plant growth and development. BR signalling is mediated by the brassinosteriod insensitive 1 (BRI1) receptor as well as non-ligand binding co-receptors of the Somatic Embryogenesis Receptor-like Kinase (SERK) family. A mathematical modelling approach was employed to assess how the co-receptors quantitatively affect the output of the BR signalling pathway. An initial model links the BRI1 receptor activity to root growth, a convenient downstream physiological readout for BR signaling. Based on the BRI1 receptor occupancy the model faithfully predicts root growth as observed in *bri1* loss-of-function mutants. At physiological ligand concentrations, only a few per cent of the total number of available BRI1 receptors are predicted to be occupied by ligand. BR signalling is robust against reduction of the BRI1 receptor level but not towards variation in the ligand concentration. This implies that signalling is mainly regulated via ligand availability and biochemical activity. Model simulations suggest that SERKs act by increasing the magnitude of the response towards BRs. When calibrated on the wild type and *serk* single mutants, the model predicts correctly that the roots of the *serk1serk3* double mutant are virtually insensitive towards BL while the hypocotyl is not. Taken together, the mathematical modelling provides additional insight into how alterations in ligand, receptor and co-receptor concentrations affect physiological responses such as root growth.

7.4 TALK 4 – SYMPOSIA 1 – 15:10–15:30

Modeling interactions between WRKY transcription factors and DNA

Nina M. Fischer[1], Luise H. Brand[2], Klaus Harter[2], Oliver Kohlbacher[3], Dierk Wanke[2]

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Question: WRKY transcription factors constitute a large protein family in plants that is involved in the regulation of developmental processes and responses to biotic and abiotic stimuli. The question arises how stimulus specific responses are mediated given that the highly conserved WRKY DNA-binding domain (DBD) exclusively recognizes the ‚TTGACY' W-box consensus sequence. Methods: *At*WRKY11 and *At*WRKY50 exhibit an amino acid difference within the highly conserved second β-strand. The conserved glutamine is changed to a lysine within the *At*WRKY50 DBD subgroup. Using molecular modeling and molecular dynamics simulations we investigated the interactions formed by glutamine in *At*WRKY11 and lysine in *At*WRKY50 with DNA. Results: Our modeling results and molecular dynamics simulations display that lysine is positioned close to the DNA phosphate backbone, whereas the mutated glutamine interacts with the thymine base. Our data helped to identify amino acids that are most probably in direct contact with the DNA indicating specificity. Conclusion: However, the mechanism by which the conserved 'GAC' core recognition is mediated remains elusive. This implies that other components are essentially required besides the W-box specific binding to DNA to facilitate a stimulus specific WRKY function.

7.5 TALK 1 – SYMPOSIA 7 – 13:30–14:00

Seeing the trees through the forest: live imaging of plant development.

Joop EM Vermeer[1]

[1]UNIL, DBMV, Lausanne, Switzerland

Live imaging is a very powerful approach to better understand developmental processes. Moreover, it also allows quantification of biological processes, especially by new microscope systems that combine speed with enhanced sensitivity. I will highlight some new developments in light microscopy that I believe are very promising for the coming years. Subsequently, I will present some results regarding the inter cell layer communication between the pericycle and endodermis during lateral root formation. Using live imaging I observed that the endodermis responds to a forming lateral root by a localized loss of cell volume. Interfering with these accommodating responses by cell type-specific expression of the stabilized AUX/IAA *shy2-2,* results in an early block in lateral root formation. I hypothesise that this results from mechanical pressure on the pericycle, blocking lateral root initiation. This suggest that accommodating responses by endodermal cells play a key role during lateral root initiation, revealing important inter-cell layer communications ignored until now. This interaction provides a unique system to study how plant cells sense and accommodate to growth of their neighbours -such "mechanical" communication might also be of importance to maintain tissue integrity in growing apical meristems or during secondary growth or root nodule formation, for example.

7.5 TALK 2 – SYMPOSIA 7 – 14:00–14:20

Cell division and morphological patterning of Marchantia analysed by live-cell microscopy of GFP-labelled microtubules

Henrik Buschmann[1], Agnes Borchers[1], Michael Holtmannspötter[1], Martin O'Donoghue[1], Sabine Zachgo[1]

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Phylogenetic analyses indicate that liverworts are the closest living relatives of the first land plants. We are interested in understanding how morphological patterning arose in the earliest land plants and how this is connected to the evolution of the cytoskeleton. The liverwort Marchantia polymorpha carries a number of important cytoskeletal novelties: it is known to exhibit preprophase bands and at the same time it shows so-called polar organizers, a specialized type of MTOC only known from liverworts. We investigated Marchantia tubulinß genes in order to establish a FP-based microtubule marker. Whereas tubulinß genes 1-3 were expressed in all tissues analyzed, tubulin ß4 was found to be male and antheridiophore specific. Expression of the GFP-Marchantia tubulinß 1 fusion by the EF1a promoter allowed live-cell imaging of various Marchantia cell types including the free swimming spermatozoids. Studies of microtubule dynamics and comparison with data from Arabidopsis suggested similar properties of interphase microtubule cortial arrays concerning self-organization. Analyses of cell division revealed the dynamic behaviour of the polar organizers and preprophase bands. To test whether microtubules control tissue patterning in Marchantia we applied microtubule drugs. The data suggest that microtubules, likely by the aid of the preprophase band, direct division plane positioning in Marchantia. The bearings of these results for the mechanism of morphological patterning of early land plants is discussed.

7.5 TALK 3 – SYMPOSIA 7 – 14:20–14:40

Leading to accuracy: Function of Phragmoplast Orienting Kinesins

Elisabeth Lipka[1], Sabine Muller[1]

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In plants proper spatial control of cytokinesis is crucial to create the cellular network of tissues. The plane of division is predicted by the preprophase band (PPB), a cortical ring of cytoskeletal filaments. This spatial information has to be retained after the PPB disassembly, by establishing the cortical division site (CDS). During cytokinesis the phragmoplast (PP) facilitates cell plate assembly. It expands from the center of the cell outwards until the forming cell plate fuses with the cortical division site exactly. Accurate execution of division site establishment as well as PP guidance is essential for cell and plant morphology. Simultaneous mutation of *Phragmoplast Orienting Kinesin (POK) 1* and *2* leads to defective cytokinesis and consequently shows disorganized cell wall pattern in roots of *Arabidopsis thaliana*. Our phenotypic characterization revealed alterations in PP expansion. Furthermore localization studies indicate association with microtubules and support POKs involvement in division site establishment and function in PP guidance.

7.5 TALK 4 – SYMPOSIA 7 – 14:40–15:00

The 2in1 system allows ratiometric BiFC and trafficking analysis

Christopher Grefen[1], Rucha Karnik[2], Mike Blatt[2]

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Binary interaction techniques are vital tools that shape our understanding of protein complexes. An inherent flaw, however, with most current protein-protein interaction techniques is the variability in expression levels for fusion proteins when using several individual plasmids. We established a novel recombination-based cloning strategy - "2in1" - that enables co-expression of fusion proteins on a cell-by-cell basis from a single plasmid. 2in1 allowed the development of a ratiometric Bimolecular Fluorescence Complementation assay (rBiFC) [1] where both candidate genes are simultaneously cloned into a single vector backbone containing an internal fluorescent marker for expression control and ratiometric analysis. rBiFC significantly increases the credibility of protein-protein interaction results allowing ratiometric comparison between different protein pairs. In addition to its use in rBiFC, 2in1 can easily be introduced into other vector systems that rely on multiple gene expression and we have successfully implemented it in secretion analysis [2], FRET and Split-Ubiquitin assays.

References

1. Grefen C, Blatt MR: A 2in1 cloning system enables ratiometric bimolecular fluorescence complementation (rBiFC). Biotechniques 2012, 53: 311-314.

2. Karnik R, Grefen C, Bayne R, Honsbein A, Kohler T, Kioumourtzoglou D et al.: Arabidopsis Sec1/Munc18 Protein SEC11 Is a Competitive and Dynamic Modulator of SNARE Binding and SYP121-Dependent Vesicle Traffic. Plant Cell 2013, 25:1368-82.

7.6 TALK 1 – SYMPOSIA 9 – 10:30–11:00

Scanning EM - the future for studying cellular ultrastructure

Chris Hawes[1], Eric Hummel[2]

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Recently,new technologies for transmission electron microscope (EM) level resolution biological imaging have been developed, based around a combination of high resolution field emission scanning electron microscopy (FEGSEM) and the serial imaging of smooth block faces of resin embedded material. These techniques are challenging traditional transmission EM approaches for 3-D microscopy such as serial sectioning and electron tomography. Firstly, focussed ion beam milling physically etches away resin from the surface of the specimen block prior to imaging. This technique permits effective "sectioning" of the block in steps as fine as 5nm. Thus, high resolution backscattered electron data can be collected from what are effectively very thin sections, making the technique comparable with EM tomography, but with the advantage that blocks can be milled a number of microns in depth. Secondly, a system has been developed where an ultramicrotome is installed in the chamber of the SEM and conventional ultrathin sections or sections as thin as 15-20 nm can be removed from the block face over many microns and discarded prior to block face imaging. Here the potential of the application of these techniques to plant material will be presented, including methodologies for contrasting resin embedded material for SEM imaging.

7.6 TALK 2 – SYMPOSIA 9 – 11:00–11:20

Are spectroscopic measurements suitable for non-invasive determination of nitrogen and anthocyanin concentrations in purple vegetables?

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Purple carrots and red cabbage yield high anthocyanin contents (AC) which is of interest for dye production. For optimal fertilization and maximal AC, it is useful to assess nitrogen (N) status and AC of the plants non-invasively. This study aimed at testing the applicability of the portable spectroscopic device Multiplex (Force-A, France). The carrots `Deep Purple' and `Purple Sun' as well as the red cabbage `Lodero' (Bejo, NL) were grown in the field. After harvest, spectroscopic measurements were taken on leaves and roots of the carrots as well as on the peeled cabbage head. Parameters expressing the AC are ANTH_RG and ANTH_RB while N content is reflected by NBI_R and NBI_G. Total N was determined by elemental analysis or Kjeldahl procedure. Anthocyanins were extracted in 80% methanol and analyzed photometrically. In purple carrots, the total AC of the roots (0.86-12.2 mg kg-1 FM) was weakly correlated to ANTH_RG and ANTH_RB measured on roots (R2=0.31-0.47) but had no correlations to the parameters assessed on leaves. NBI_G and NBI_R from leaf or root measurements were not correlated to total N in roots. In red cabbage, ANTH_RG and ANTH_RB were not correlated to the total AC (R2=0.14). The correlation of total N with N parameters was weak (R2=0.42-0.46). Thus, the spectroscopic device Multiplex is of limited use for non-invasive determination of N status and AC in red cabbage and purple carrots.

7.6 TALK 3 – SYMPOSIA 9 – 11:20–11:40

NMR based functional imaging in living plant

Ljudmilla Borisjuk[1], Hardy Rolletschek[1], Johannes Fuchs [1,2], Eberhard Munz [1,2], Gerd Melkus[3], Thomas Altman[1], Peter Jakob [2,4]

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A major feature of magnetic resonance imaging (MRI) is that it is a non-invasive platform, and thus can be used for the analysis of living organisms. Our presentation highlights technological developments in MRI, which are creating opportunities for the visualization and quantification of structure and metabolism in plant (Borisjuk et al, Plant J 70, 2012). Description of the various analytical modes is provided and an explanation of how MRI can be applied to plant samples and what it can achieve. Various metabolite maps and dynamic images are included to demonstrate the potential of MRI and to exemplify recent cutting-edge advances in the field. Among them are dynamic imaging of germinating rapeseeds, translocation of sucrose between two plant generations, accumulation of alanine in hypoxic region of cereal endosperm, 3-dimensional images of lipid deposition in seeds and fruits of crops and model plants (Borisjuk et al., Prog Lipid Res 52, 2013). The importance and prospects of the imaging of metabolites in living plants, as well as the integration of lipid imaging with metabolic modelling (Borisjuk, Plant Cell *in press*) and other emerging techniques, as for example the matrix-assisted laser desorption/ionization-MS imaging (Horn et al, Plant J *in press*) and Synchrotron- x-ray tomography (Verboven et al, New Phytologist *in press*), are outlined to provide impetus for future application in biotechnology and plant research.

7.6 TALK 4 – SYMPOSIA 9 – 11:40–12:00

Biochemical traits of plant cells measured by FTIR spectroscopy

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In plant ecology research the focus of plant traits shifting more and more towards chemical and biochemical cell composition. Since, the analysis of elemental cell quota or cellular content in proteins, major storage compounds like lipids and carbohydrates or structural components like lignin by means of biochemical methods is time consuming and needs relative high amounts of cell material, alternative measurements are recently under development. Fourier transformed infrared spectroscopy (FTIR) can overcome some of these limitations due to its high reliability, sensitivity, and the potential for high throughput analysis. We show that FTIR spectroscopy coupled to several statistic based data interpretation is a useful tool for determining biochemical traits of different plant material, ranging from small single cell phytoplankton up to higher plant samples. We will discuss the application of the method in different fields of plant ecology and biotechnology with respect to statistical data interpretation.

8.1 TALK 1 – SYMPOSIA 1 – 14:00–14:30

Assembly of photosystem II

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Cyanobacteria, algae and plants can convert light energy to chemical energy using a very similar type of photosynthetic membrane system, named thylakoids. Current molecular analyses suggest that the initial steps underlying the biogenesis of the cyanobacterial energy conversion system, in particular photosystem (PS) II, progress in a membrane subfraction representing a biosynthetic centre with contact to both plasma and thylakoid membranes [1]. This special membrane fraction is defined by the presence of the PS II assembly factor PratA and therefore was named PDM fraction (PratA defined membrane). Recent data indicate that this factor binds and delivers manganese (Mn) to PS II and, consequently, is involved in the assembly of its water splitting Mn4CaO5 cluster [2]. The concept of thylakoid membrane biogenesis centres will be discussed with regard to its evolutionary development.

[1] Nickelsen, J. and Rengstl, B. (2013) Photosystem II assembly: From cyanobacteria to plants. Annu. Rev. Plant Biol. 64, 609-635.

[2] Stengel, A., Gügel, I., Hilger, D., Rengstl, B., Jung, H. and Nickelsen, J. (2012) Initial steps in photosystem II assembly and preloading with manganese take place in biogenesis centers in *Synechocystis.* Plant Cell 24, 660-675.

8.1 TALK 2 – SYMPOSIA 1 – 14:30–14:50

The impact of ferredoxin:NADP(H) reductase on electron partitioning

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In the final stages of the linear photosynthetic electron transport (PET) chain, photosystem I reduces ferredoxin (Fd), which then transfers these electrons to Fd:NADP(H) oxidoreductase (FNR). From this point, electrons can be released into stromal metabolism for use in reductive metabolism (as either NADPH or reduced Fd). In the absence of appropriate acceptors, these electrons may also be donated to O2, forming damaging reactive oxygen species (ROS). An alternative possibility is their return to the PET chain, in a cyclic electron transport, allowing build-up of ΔpH (and therefore ATP synthesis) without the need for a soluble electron sink. The actions of Fd and FNR are therefore at a pivotal point between generation of electrons in PET, and their use as either fuel for metabolism, sources of ROS, or their re-cycling into the PET chain. We have previously isolated transgenic plants with FNR enriched at variable locations on the thylakoid membrane1, and we will present new data from investigations into how these different FNR locations affect the fate of photosynthetically generated electrons.

1Twachtmann *et al*. (2012) Plant Cell 24(7), 2979-2991

8.1 TALK 3 – SYMPOSIA 1 – 14:50–15:10

Photo-protective mechanisms under light stress conditions: Psbs Protein Interactions During Non-Photochemical Quenching

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The non-photochemical quenching of excitation energy (NPQ) describes a photoprotective mechanism in the antenna of PSII which dissipates excess excitation energy as heat at the level of 1Chl* and by that prevents the formation of singlet oxygen in PSII. Four different components contribute to NPQ, namely qT, qE, qZ and qI. Under saturating light conditions, the qE represents the dominant NPQ component; qE is based on a complex mechanism which strictly depends on the ΔpH across the thylakoid membrane, the PsbS protein and the xanthophyll zeaxanthin (Zx). According to the current understanding of qE, a low pH in the thylakoid lumen induces (i) PsbS-dependent conformational changes in the antenna of PSII and (ii) the formation of Zx, resulting in the quenching of excitation energy at the PSII antennae. The central role of PsbS in these processes is related to the function of PsbS as sensor of the lumen pH. However, the molecular basis of this central function and particularly the underlying interactions of PsbS with PSII antenna proteins that lead to energy quenching are largely unclear. In this work, we present an *in vitro* approach to identify protein transient interactions involving the PsbS protein during NPQ induction and relaxation in *Arabidopsis thaliana*, revealing its direct role in the formation of quenching sites in the antenna complexes of photosystem II.

8.1 TALK 4 – SYMPOSIA 1 – 15:10–15:30

The *hcf107* mutation of *Arabidopsis thaliana* can be complemented by a compartment-alien transformation with the plastome-encoded *psbH* gene

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High chlorophyll fluorescence-mutants of *Arabidopsis thaliana* are used to identify factors, which are important for the biogenesis of PSII. In one of these mutants, *hcf107,* PSII subunits are strongly reduced, especially PsbH and CP47 (PsbB). Consequently, the *hcf107* mutant is not able to grow photoautotrophically. Both of these PSII subunits are encoded by plastid genes and are part of the polycistronic *psbB*-operon. The lack of PsbH correlates with the absence of those RNAs in which *psbH* is the leading cistron, indicating that only these RNAs are fundamental for PsbH translation. In contrast, the amount of *psbB* RNA is not affected. This raises the question, if the reduction of CP47 protein is a secondary affect due to the absent PsbH. To test this hypothesis we attempted to provide PsbH protein to *hcf107* chloroplasts by equipping the gene with the plastidial targeting sequence of the PsbS protein and the 35S promoter. This chimeric gene was inserted into the genome of the *hcf107* mutant. We found that the nuclear-localised chimeric *psbH* gene was able to complement the mutant defect resulting in plants that grew photoautotrophically. Further experiments showed that cytosolically synthesized PsbH protein was assembled into PSII complexes. This is the first time in which a defect in a plastome-encoded hydrophobic membrane protein could be rescued by a compartment-alien transformation with that gene.

8.2 TALK 1 – SYMPOSIA 4 – 14:00–14:30

C4 photosynthetic promoters use a histone code to integrate developmental and environmental stimuli

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[1]Gottfried Wilhelm Leibniz Universität Hannover, Institute of Botany, Hannover, Germany

C4 photosynthesis evolved more than 60 times independently. One major aspect of this process was a recruitment of existing non-photosynthetic genes into the pathway. The promoters of these genes had to acquire numerous new regulatory features such as high expression, light induction, tissue specificity, and response to metabolic stimuli. All these different inputs had to be integrated by the promoters into a single, but tunable, output, the rate of transcription. We used the promoter of the core C4 gene phosphoenolpyruvate carboxylase as a model to study the underlying processes. Our analyses revealed that specific histone modifications were associated with each individual stimulus. Interpretation of the modification was influenced by the promoter position. Comparative analyses with the promoters of other C4 genes in maize and further C4 grasses revealed a high degree of conservation of this code, even if the species did not share a common C4 ancestor. Moreover, other genes that were not part of the C4 mechanism also used the code. These results indicated that a pre-exisiting histone code for gene regulation was used during the evolution of C4 metabolism. In this presentation, we will show how the individual elements of the code were defined. We will also report on our progress in identifying the methyltransferases and acetyltransferases writing the code.

8.2 TALK 2 – SYMPOSIA 4 – 14:30–14:50

Loss of Cytosolic Phosphoglucose Isomerase (cPGI) affects carbohydrate metabolism in leaves and is essential for fertility of Arabidopsis thaliana

Shirin Zamani-Nour[1], Hans-Henning Kunz[1], Rainer Häusler[1], Julian I. Schroeder[2], Irina Malinova[3], Jörg Fettke[3], Ulf-Ingo Flügge[1], Markus Gierth[1]

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[3]University of Potsdam, Biochemistry and Biology, Potsdam, Germany

Phosphoglucose isomerases (PGI) are key enzymes in carbohydrate metabolism involved in starch and sucrose biosynthesis. Two isoenzymes are encoded in the *Arabidopsis* genome, one of which is located in plastids and one in the cytosol. The starch-free loss-of-function mutant of the plastidic isoform (*pgi1*) has been analyzed in detail. However, corresponding mutants of the cytosolic PGI (*cpgi*) have not been investigated yet. The transmission efficiency of *cpgi* T-DNA insertion alleles was strongly decreased through both male and female gametophytes and homozygous T-DNA insertion mutants were nonviable. Interestingly, plants expressing artificial micro RNAs targeting *cPGI* mRNA under control of the CaMV 35S promoter were vital although cPGI activity was not detectable in leaves of mature plants. amiRNA *cpgi* plants were significantly reduced in size, displayed starch excess in leaves as well as a higher glucose content of soluble cytosolic heteroglycans and reduced sucrose content in leaf tissue at the end of the night. Moreover, increased non photochemical quenching in amiRNA leaves indicated an impact of absent cPGI activity on photosynthesis. Taken together, our data provide evidence for a vital role of cPGI during reproduction while loss of cPGI activity can apparently be compensated in mature plants but strongly affects nocturnal starch breakdown and impacts photosynthesis.

8.2 TALK 3 – SYMPOSIA 4 – 14:50–15:10

Transcriptional response of *A. thaliana* wild type and photorespiratory mutants towards changes in CO2 levels

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Photorespiration is an essential process in oxygenic photosynthetic organisms but also reduces overall productivity. Accordingly, the underlying photorespiratory cycle has been studied intensively. In contrast to the core metabolism little is known about intertwining pathways. To identify and characterize these associated metabolic pathways and also regulatory mechanisms we performed a comparative transcriptomic analysis of *Arabidopsis thaliana* wild type (WT) plants shifted from 1 % CO2 conditions to ambient air conditions with 0.039 % CO2. We found that the WT shows a very mild transcriptional response 8 h after the shift with changed expression levels for only 1.5 % of all genes. Remarkably, transcription of photorespiratory genes was not significantly affected. In contrast to the WT mutants in the photorespiratory cycle showed a strong transcriptional response. At an average 20 % of the genes were differentially expressed in the mutants compared to WT after the change in CO2 levels. Here, we describe and discuss which compensatory mechanisms the mutants employ to cope with the consequences of interrupted photorespiratory metabolism.

8.2 TALK 4 – SYMPOSIA 4 – 15:10–15:30

Time and light dependent regulation of the Calvin Cycle in the diatom *Phaeodactylum tricornutum*

Matthias Sachse[1], Sabine Sturm[1], Ansgar Gruber[1], **Peter Kroth**[1]

[1]University of Konstanz, Department of Biology, Konstanz, Germany

Diatoms are unicellular algae that evolved by secondary endosymbiosis which had a strong impact on the metabolism and its regulation. While light induced redox-regulation of the Calvin Cycle is a central feature in higher plants, the capacity for such redox-regulation is strongly reduced in diatoms. We have investigated the light and time depending regulation of the key enzymes of the Calvin Cycle in the diatom *Phaeodactylum tricornutum* on the transcriptional and protein level. We determined transcript and protein levels over time under two different light conditions in order to distinguish between diurnal effects and light-dependent changes. We were able to demonstrate a surprisingly strong transcriptional regulation for both the phosphoribulokinase (PRK) (light modulated diurnal regulation) and plastidic glyceraldehydes-3-phosphate dehydrogenase (GAPC1) (primarily light independent diurnal regulation), which is exceeding any relative changes which are known for higher plants under similar light and time conditions by far. Other investigated Calvin Cycle genes show a light modulated diurnal control of weak to moderate magnitude. There is evidence that blue light may affect transcription. We therefore have studied the role of aureochromes, blue light photoreceptors that are specifically found in stramenopile algae.

8.3 TALK 1 – SYMPOSIA 6 – 10:30–11:00

Quantitative analysis of metabolism on a network scale unlocks the complexity of central carbon metabolism

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Plant central metabolism is known for its remarkable redundancy and overlap in functionality of its components. There is increasing awareness that future improvement of our understanding of control and functioning of central metabolism relies on in-vivo biochemical quantification on a network scale, which includes metabolic flux analysis. Here I like to highlight some current developments in methods of stationary and instationary metabolic flux analysis in plants and its relation to other emerging modeling approaches like Flux Balance Analysis. In addition, recent insights from flux analysis into pathway usage and functionality in developing oilseeds from different species will be summarized. An example of integration of flux with other quantitative data will be discussed along with a perspective on predictive models.

8.3 TALK 2 – SYMPOSIA 6 – 11:00–11:20

Connecting the Peroxisomes - metabolite transporters across the Peroxisome membrane

Nicole Linka[1], Kristin Bernhardt[1], Sarah K. Kessel-Vigelius[1], G. Schroers[1], Jan Wiese[1], Thomas J. Wrobel[1], Andreas P.M. Weber[1]

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Plant peroxisomes are essential organelles, which play important roles in plant growth and development, throughout the life cycle of the plant. They house biochemical reactions involved in lipid metabolism, photorespiration, synthesis of signalling molecules and secondary products. They are centrally placed in cellular metabolism, sharing several metabolic pathways with other compartments, such as plastids and mitochondria. To connect peroxisomes with other cell compartments, specific transport proteins are required to enable the flux of metabolites. Given the large number of substrates that are exchanged across the peroxisomal membrane, a wide spectrum of transporters is expected. Although great progress has been made in peroxisome biology through genomics and proteomics, very little is understood regarding transporter proteins in the peroxisome membrane. Our group has discovered plant peroxisomal carrier proteins mediating the import of the cofactors ATP, NAD and CoA required for a wide range of metabolic reactions inside peroxisomes. We will give a detailed update on the diverse functions of these cofactor transporters in plants. In addition we will present a novel metabolite carrier of plant peroxisomes involved in exchange of small organic acids.

8.3 TALK 3 – SYMPOSIA 6 – 11:20–11:40

Seed architecture shapes embryo metabolism in oilseed rape

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In this work, we investigate how central metabolism of the growing embryo of oilseed rape is adjusted to developmental changes in its architecture (Borisjuk et al., Plant Cell, in press). Non-invasive nuclear magnetic resonance based imaging of seed illustrates that, following embryo bending, gradients in lipid concentration became established. These were correlated with the local photosynthetic electron transport rate, and the accumulation of storage products. Experimentally induced changes in embryo morphology and/or light supply altered these gradients, and were accompanied by alterations in both proteome and metabolome. Flux balance analysis predicted that the outer cotyledon and hypocotyl/radicle generate the bulk of plastidic reductant/ATP via photosynthesis, while the inner cotyledon, being enclosed by the outer cotyledon, is forced to grow essentially heterotrophically. Under field-relevant high-light conditions, major contribution of the RubisCO-bypass to seed storage metabolism is predicted for the outer cotyledon and the hypocotyl/radicle only. We conclude that invivo metabolic fluxes are locally regulated and connected to seed architecture, driving the embryo towards an efficient use of available resources such as light and space.

Borisjuk L, Neuberger T, Schwender J, Heinzel N, Sunderhaus S, Fuchs J, Hay JO, Tschiersch H, Braun HP, Denolf P, Lambert B, Jakob PM, Rolletschek H. (2013): Seed architecture shapes embryo metabolism in oilseed rape. Plant Cell (manuscript in press).

8.3 TALK 4 – SYMPOSIA 6 – 11:40–12:00

A systems biology analysis of early-stage sugar beet storage root development

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In sugar beet (*Beta vulgaris* L.), sucrose is not only the major transport form of assimilates, but it also accumulates at high concentrations in storage roots. Since sugar beet studies so far concentrated on source-sink relations in mature plants with a fully developed storage root, the metabolic changes occurring during the initial phase of sugar beet storage root development have not been systematically addressed. In the presented study, a systems biology analysis of early-stage sugar beet storage root development was conducted. The activities for different key enzymes of carbohydrate metabolism were analyzed in developing storage roots over the first 80 days after sowing, complemented with an *in situ* localisation of selected enzyme activities, expression analyses for the respective transcripts, anatomical investigations, and soluble sugar, hexose-phosphate and phytohormone profiles. Based on the accumulation dynamics of biomass and sucrose, as well as on anatomical parameters, the early phase of storage root development can be subdivided into two stages (prestorage stage; secondary growth and sucrose accumulation stage), each of which is characterised by distinct metabolic, transcriptional and phytohormonal signatures. The onset of secondary growth and sucrose storage is preceded by a phase of metabolic transition.

8.4 TALK 1 – SYMPOSIA 9 – 10:30–11:00

Integration of organ-specific biosynthesis and long-distance transport establish distinct glucosinolate profiles in vegetative Arabidopsis

Barbara Ann Halkier[1]

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In plants, transport processes are important for the reallocation of defense compounds to protect tissues of high value, as exemplified in the model plant Arabidopsis where the major defense compounds, glucosinolates, are translocated to seeds upon maturation. We have identified and characterized two members of the NRT/PTR family, AtGTR1 and AtGTR2, as high-affinity, proton-dependent glucosinolate-specific transporters. The *Atgtr1 Atgtr2* double mutant did not accumulate glucosinolates in the seeds and had over-accumulation in leaves and silique walls, which shows that both AtGTR1 and AtGTR2 are essential for long-distance transport of glucosinolates to the seeds. Reciprocal grafting experiments indicated that both rosette and roots are source tissue for aliphatic and indole glucosinolates, and that GTR1 and GTR2 play a role in controlling the distribution of aliphatic, but not indole glucosinolates through long-distance transport in both the phloem and xylem in Arabidopsis plants at the vegetative stage. Our work shows that the specific distribution patterns of glucosinolates between rosette and root in Arabidopsis are formed by integration of long-distance transport through the vasculature and specific above- and belowground de novo biosynthesis.

Nour-Eldin HH et al. (2012) Nature. 488, 531-534.

8.4 TALK 2 – SYMPOSIA 9 – 11:00–11:20

Biochemistry of Glucosinolate Breakdown: Towards a better understanding of Specifier protein structure and Function

Frauke Gumz[1], Leif Barleben[1], Ute Wittstock[1]

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Specifier proteins are part of the glucosinolate-myrosinase defense system of the Brassicaceae. Upon tissue damage, myrosinases hydrolyse glucosinolates to toxic isothiocyanates. In the presence of specifier proteins, breakdown is redirected to alternative products such as nitriles, epithionitriles or thiocyanates. Question: How do specifier proteins work biochemically? Methods: A purification protocol was established for recombinant Arabidopsis thaliana nitrile-specifier protein (AtNSP3-His6) and thiocyanate-forming protein from Thlaspi arvense (TaTFP, GST-tag removed) to obtain sufficiently pure protein for structure elucidation and interaction studies with purified Sinapis alba myrosinase using Mts-Atf-Biotin as a photoactivatable trifunctional crosslinker. Results: TaTFP has been purified to homogeneity at 2 mg/ml and is being used for crystallography. Incubation of Mts-Atf-Biotin-labeled myrosinase with AtNSP3-His6 or TaTFP at 366 nm resulted in crosslinking and label transfer after DTT treatment. Deletion of the lectin-domain of AtNSP3 did not interfere with label transfer. Conclusion: Myrosinase interacts with specifier proteins at a distance of <11.1Å, and this interaction is independent of lectin-domains. Experimental structure elucidation has become a realistic possibility.

8.4 Talk 3 – Symposia 9 – 11:20–11:40

In *Arabidopsis thaliana* small subunit 1 of the isopropylmalate isomerase is required for leucine and glucosinolate biosynthesis as well as leaf patterning

Janet Imhof[1], Michael Reichelt[2], Jonathan Gershenzon[2], Kurt Lächler[1], **Stefan Binder**[1]

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In *Arabidopsis thaliana* the evolutionary and functional relationship between Leu biosynthesis and the Met chain elongation pathway, the first part of glucosinolate formation, is well documented. But still the exact functions of some pathway components are unclear. Isopropylmalate isomerase (IPMI), an enzyme normally involved in Leu biosynthesis, is a heterodimer consisting of a large and a small subunit. While the large protein is encoded by a single gene (*IPMI LSU*), three genes encode small subunits (*IPMI SSU1 to 3*). We now analyzed small subunit 1 of IPMI using an artificial microRNA approach. Strong reduction of IPMI SSU1 mRNA levels resulted in a severe phenotype with stunted growth, narrow pale leaf blades, abnormal flower morphology and abnormal adaxial-abaxial patterning. Supplementation the knockdown plants with Leu could only partially compensate the morphological and developmental abnormalities. A detailed metabolite profiling of the knockdown plants revealed changes in the steady state levels of isopropylmalate and glucosinolates as well as its intermediates demonstrating a function of IPMI SSU1 both in Leu biosynthesis and the first cycle of Met chain elongation. Surprisingly the levels of free Leu slightly increased suggesting an imbalanced distribution of Leu within cells and/or within plant tissues.

8.4 TALK 4 – SYMPOSIA 9 – 11:40–12:00

Transcritpomics of selected pyrrolizidine alkaloid producing plants

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Pyrrolizidine alkaloids (PAs) are produced by several plant species from distantly related families like Asteraceae, Boraginaceae or Orchidaceae as repellent against herbivores. One well characterized step in plant PA biosynthesis is catalyzed by the homospermidine synthase (HSS). It was shown that HSS evolved at least five times independently in plant kingdom. How the complete pathway was established during evolution is still far from being understood. HSS expression is restricted to specialized cell types. This specificity was utilized in order to identify gene transcripts of HSS expressing cells using a combination of cDNA subtraction and 454-sequencing. Roots of *Eupatorium cannabinum* L. (Asteraceae), leaves of *Symphytum officinale* L. (Boraginaceae) and single epidermic cells of *Heliotropium indicum* L. (Heliotropiaceae) have been analyzed. Cell specific specimen of *H. indicum* have been prepared with laser capture microdissection enabling a view on gene expression at cellular level. Several identified candidate genes of PA biosynthesis and their evolution will be discussed.

Session 8.5 – Transport Processes: Regulation and Impact on Plant Performance/Properties

Wednesday 10/02 – 15:30-17:00 – Chair: Nicolaus von Wirén

8.5 TALK 1 – SYMPOSIA 8 – 15:30–16:00

Regulation of ammonium transport and sensing in plant roots

Nicolaus von Wirén[1]

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Together with nitrate ammonium represents the most important nitrogen source for the nutrition of plants. High-affinity uptake of ammonium by plant roots is mediated by a family of AMMONIUM TRANSPORTER (AMT)-type transport proteins, in which individual members differ in biochemical properties and cell type-specific localization to generate an overall transport capacity that is tightly regulated by nitrogen at different regulatory levels. Besides acting as a nutrient, external ammonium is perceived by plant roots as a signal. Ammonium shuts off AMTs by C-terminal phosphorylation leading to trans-inactivation of neighbouring subunits in a trimeric AMT protein complex. In addition, ammonium sensing displays at the morphological level. This is based on the observation that localized ammonium supply enhances lateral root branching, to which individual AMTs contribute to a different extent. These AMT-dependent changes in root morphology are reminiscent of ammonium-induced morphological changes in hyphal structures of yeast and fungi, emphasizing common features in ammonium signaling.

8.5 TALK 2 – SYMPOSIA 8 – 16:00–16:20

Sugar transport across the vacuolar membrane

Ekkehard Neuhaus[1]

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Carbohydrates fulfill many important functions as energy reserves, as inter-organ energy transport molecules, as osmolytes required to survive phases of drought or cold, as precursors for starch and cellulose biosynthesis or as chemical groups necessary for protein and lipid modification. Sugar accumulation occurs intracellular in the large vacuole. Within this presentation the vacuolar monosaccharide transporter TMT and its regulation will be introduced (Wingenter *et al.*, 2010, Wingenter *et al.*, 2011). In addition, the function of two so far uncharacterized vacuolar monosaccharide exporters belonging to the SWEET family will be clarified (Chadron *et al.*, 2013). With these discoveries a more complete picture on the dynamics of vacuolar sugar metabolism can be drawn.

Chadron F, et al. 2013. Leaf fructose content is controlled by the vacuolar transporter SWEET17 in Arabidopsis. Curr.Biol., in press.

Wingenter K, et al 2010. Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signalling and seed yield in Arabidopsis. Plant Physiol. 154: 665-677.

Wingenter K, et al. 2011. A member of the mitogen-activated protein 3-kinase family is involved in the regulation of plant vacuolar glucose uptake. Plant J 68: 890-900.

8.5 TALK 3 – SYMPOSIA 8 – 16:20–16:40

PLGG1, a novel plastidic glycolate glycerate transporter, is essential for photorespiration

Thea Renate Pick[1], Andrea Bräutigam[1], Toshihiro Obata[2], Alisdair R. Fernie[2], Andreas P.M. Weber[1]

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Although Rubisco is the most abundant protein on earth, it is inefficient due to its catalytic activity to both carboxylate and oxygenate the acceptor ribulose-1,5-bisphosphate. The products of the oxygenation reaction are 3-PGA and 2-PG. 2-PG is toxic to plants and has to be detoxified in the photorespiratory cycle with metabolic fluxes reaching up to one third of photosynthetic fluxes. Photorespiration is a compartmentalized process with enzymatic reactions taking place in chloroplasts, peroxisomes, mitochondria and the cytosol. Although all soluble enzymes involved in this pathway have been characterized, no transporter of the core cycle has been identified on the molecular level to date. Here we report the molecular identification of the first transporter involved in the core photorespiratory cycle. A reverse genetics approach identified Plastidic glycolate glycerate transporter (PLGG1) as a candidate. An *Arabidopsis thaliana* mutant deficient in PLGG1 shows a photorespiratory phenotype. Glycolate and glycerate, both metabolites of the photorespiratory pathway, accumulate in *plgg1-1* plants. Finally, *in vivo* and *in vitro* transport assays indicate that glycolate and glycerate transport are impaired in the mutant. These results show that PLGG1 is the plastidic glycolate/glycerate transporter involved in the core photorespiratory cycle.

8.5 TALK 4 – SYMPOSIA 8 – 16:40–17:00

FAX1, a novel membrane protein in the chloroplast inner envelope mediates export of fatty acids

Katrin Philippar[1], Nannan Li[1], Irene Gügel[1], Jürgen Soll[1]

[1]LMU München, Department Biologie I, Biochemie & Physiologie der Pflanzen, Planegg, Germany

Plastids harbor many vital biosynthetic functions during growth and development. Thus, plastid metabolite synthesis requires solute exchange across the outer and inner envelope membranes. Because fatty acid synthesis in plants exclusively takes place in plastids, export for further lipid metabolism is required. However, knowledge on proteins involved in plastid export of fatty acids is still scarce. We selected FAX1 (fatty acid export 1), a novel membrane-spanning protein in *Arabidopsis* chloroplasts. According to GFP-targeting and immunoblot analysis, FAX1 spans the plastid inner envelope membrane. *FAX1* knockout mutants show reduced biomass, thin inflorescence stems and strongly impaired male fertility. In contrast, *FAX1* overexpression leads to increased biomass and thicker stems. Transcriptomic, metabolic and ultrastructural analysis imply that FAX1 is a key protein for synthesis of secondary metabolites, such as pollen exine layers or epidermal waxes, which both require plastid fatty acid export. Further, leaves and flowers of *FAX1* k.o. show reduced phospholipid and triacylglycerol contents, respectively. *FAX1* overexpressors again behave *vice versa* and show increased TAG levels. Since FAX1 could restore uptake of α-linolenic acid into a fatty acid transport-defective yeast mutant, we propose a function of FAX1 in export of fatty acids and/or derivatives from plastids.

9.1 TALK 1 – SYMPOSIA 1 – 14:00–14:30

Different light harvesting systems in diatoms and their involvement in non-photochemical quenching

Claudia Büchel[1]

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Diatoms can be divided in two principal groups, the centrics and pennates. Both possess membrane intrinsic light harvesting antenna, the fucoxanthin chlorophyll proteins (FCP). Their absorbance is dominated by their high amount of fucoxanthin and by Chl c. Three groups of FCP proteins can be found in both diatom groups: lhcf for the main light harvesters, lhcr for photosystem I antennae proteins and lhcx for FCPs assumed to be involved in photoprotection due to non-photochemical quenching (NPQ). Despite these similarities, the overall arrangement of FCP proteins differs between the groups. In pennate diatoms only trimeric complexes were found so far, built solely of Lhcf proteins. The localisation of lhcx is still under debate. In contrast, in centric species the trimeric FCPa complexes consist of Lhcf and Lhcx proteins. In addition, FCPb complexes of higher oligomeric state and different polypeptide composition (Lhcf) are present. The content of Lhcx proteins in FCPa correlates with the level of diatoxanthin synthesized under NPQ conditions and its content was inversely correlated with the fluorescence yield of FCPa. Low pH values, influencing NPQ *in vivo,* were also shown to reduce its fluorescence yield, as was aggregation of FCPa. In addition, one red shifted fluorescence band of low yield could be identified only in aggregated FCPa. A model for the involvement of FCPs in NPQ is presented.

9.1 TALK 2 – SYMPOSIA 1 – 14:30–14:50

Turning the tables: Increasing the photorespiratory capacity in *C. reinhardtii* for biogas production

Anja Günther[1], Theresa Quaas[1], Torsten Jakob[1], Reimund Goss[1], Christian Wilhelm[1]

[1]University of Leipzig, Plant Physiology, Leipzig, Germany

Most of the current approaches to gain biofuels by microalgae base upon biomass production. However, to date, the energetic costs for further biomass processing exceed any positive energy balance of algal biomass production. We established a new, non-biomass-based approach focusing on the excretion of glycolate as a substrate for biogas production [Günther et al. 2012]. Glycolate is an early product of the photorespiration. In *C. reinhardtii,* it is synthesized under high O2 conditions and either recycled in the C2 cycle or directly excreted into the medium. In conventional approaches it is attempted to avoid photorespiratory activity since the related loss of carbon significantly decreases the efficiency of biomass production. We compared different *C. reinhardtii* strains by analyzing their capacity to accumulate biomass during photosynthesis and their ability to excrete glycolate under photorespiratory conditions. Therefore, the cells were first grown under high CO2 and subsequently shifted to low CO2 and high O2. To quantify photosynthesis, we applied pulse amplitude modulation fluorescence combined with oxygen evolution and dry weight measurement. Glycolate was determined based on a colorimetic test [Takahashi, 1972]. As a result, we established different strategies to increase and stabilize glycolate excretion by optimizing physiological conditions and by genetic modulation of key steps of the cell metabolism.

9.1 TALK 3 – SYMPOSIA 1 – 14:50–15:10

Studying xanthophyll cycle pigments and Lhcx proteins in the diatom *Phaeodactylum tricornutum*: More than 'just' NPQ

Bernard Lepetit[1,2], Sabine Sturm[1], Alessandra Rogato[3,4], Gautier Gelin[2], Mariana Lepetit[2], Ansgar Gruber[1], Matthias Sachse[1], Angela Falciatore[4], Peter G. Kroth[1], Johann Lavaud[2]

[1]Universität Konstanz, Biologie, Konstanz, Germany
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[3]Institute of Genetics and Biophysics, Naples, Italy
[4]CNRS-Université Pierre et Marie Curie, Paris, France

The major photoprotective mechanism of diatoms is called 'Non Photochemical Fluorescence Quenching' (NPQ). qE, its most prominent part, needs the conversion of the xanthophyll diadinoxanthin (Dd) into diatoxanthin (Dt) during illumination and the presence of nuclear-encoded Lhcx antenna proteins. We used the diatom *P. tricornutum* to investigate its acclimation to different light climates regarding the expression of *lhcx* genes and the amount of Dd+Dt. Dd+Dt pigments and *lhcx1*, *lhcx2* and *lhcx3* were up regulated by high light (HL) intensities, but the respective *lhcx* transcript changes were variable depending on the specific light conditions. To investigate the actual trigger of *lhcx* and Dd+Dt up-regulation, several photosynthetic inhibitors were applied. By modifying the redox state of the plastidic plastoquinone (PQ) pool with DCMU or DBMIB, we achieved to stop the Dd+Dt synthesis at HL as well as its stimulation at low light (LL), respectively. Moreover, an induction of *lhcx1* and *lhcx2* gene expression by a reduced PQ pool (with DBMIB) at LL conditions was reached. These results reveal a highly complex photoprotective strategy of diatoms and indicate a key role of the redox state of the PQ pool in triggering light acclimation responses. Also, they suggest the presence of a plastid-to-nucleus retrograde signaling mechanism in an organism with secondary endosymbiosis derived chloroplasts.

9.1 TALK 4 – SYMPOSIA 1 – 15:10–15:30

HSF1 Controls Circadian Rhythmicity and Ambient Temperature-Regulation under the Influence of CK1 in *Chlamydomonas reinhardtii*

Jens Boesger[1], Olga Weisheit[1], Daniela Strenkert[2], Michael Schroda[2], Maria Mittag[1]

[11]Institute of General Botany and Plant Physiology, Friedrich Schiller University Jena, Jena, Germany
[2]Department of Molecular Biotechnology & Systems Biology, University of Kaiserslautern, Kaiserslautern, Germany

HEAT SHOCK FACTOR 1 (HSF1) of the green flagellate alga *Chlamydomonas reinhardtii* induces the expression of heat shock protein genes like *HSP70A* or *HSP90A* after exposing cells to 40°Ca. Here we show that HSF1 is also an important regulator of the circadian clock of *C. reinhardtii*, as indicated by period-shortening in HSF1 knock-down strains. HSF1 is also involved in adapting the expression levels of the clock-relevant *CASEIN KINASE1* (*CK1)*b, c gene to variations in ambient temperatures (18°C versus 28°C). Chromatin immunoprecipitation assays showed that HSF1 does not bind to the *CK1* promoter. However, it binds to the *HSP90A* promoter and this binding is increased in cells grown at 28°C as compared to 18°C. Treatment of cells with geldanamycin, a specific HSP90A inhibitor, abolishes the temperature-dependent up-regulation of CK1. Immunoprecipitation assays revealed that HSP90A interacts with CK1. These data indicate that HSP90A acts as a mediator for the regulation of CK1 levels by HSF1. aSchulz-Raffelt et al., 2007, Plant J. 52: 286-295; bSchulze et al., 2013, Mol Plant. 6:931-44; cSchmidt et al., 2006, Plant Cell 18: 1908-1930.

Session 9.2 – Biodiversity and Ecological Functions of Algae and Seagrasses **Wednesday 10/02 – 10:30-12:00 –** Chair: Thorsten Reusch

9.2 TALK 1 – SYMPOSIA 6 – 10:30–11:00

Evolutionary adaptation to global change in marine photoautotrophic microbes

Thorsten Reusch[1]

[1]GEOMAR, Evolutionary Ecology, Kiel, Germany

Global climate change in the oceans is associated with the simultaneous change of several environmental parameters such as warming, acidification and nutrient supply. It thus has the potential to dramatically change plant physiology, community composition, and may result in adaptive evolution. My talk focuses on photoautotrophic microbes (phytoplankton and microphytobenthos) which are responsible for ~50% of global primary productivity. Although their large population sizes, standing genetic variation and rapid turnover time should promote swift evolutionary change, marine biologists have only recently begun to address the possibility of evolutionary adaptation. I present case studies from a coccolithophore species (*Emiliania huxleyi*) as member of calcifying marine phytoplankton, where experimental evolution revealed adaptation to enhanced partial pressure of CO2 via lineage sorting (clonal selection) and de novo mutations. As an alternative approach, comparisons of genotypes isolated from contrasting habitats can be utilized to infer local adaptation along with controlled laboratory exposures. This approach is currently evaluated near a Mediterranean vent site off Sicily using benthic diatom species isolated from sites that are naturally enriched in CO2 versus control isolates.

9.2 TALK 2 – SYMPOSIA 6 – 11:00–11:20

Contribution of Water Framework Directive (WFD) monitoring to scientific biodiversity research

Rolf Karez[1], Inka Bartsch[2], Karin Fürhaupter[3], Ralph Kuhlenkamp[4], Verena Sandow[5], Philipp Schubert[6]

[1]State Agency for Agriculture, Environment and Rural Areas Schleswig-Holstein (LLUR SH), Coastal Waters, Flintbek, Germany
[2]Alfred-Wegener-Institute, Bremerhaven, Germany
[3]MariLim, Schönkirchen, Germany
[4]Phycomarin, Hamburg, Germany
[5]Coastal Research Management, Kiel, Germany
[6]GEOMAR, Kiel, Germany

The European Water Framework Directive requests the monitoring of marine macroalgae and angiosperms ("macrophytes") and, thus, guarantees the regular acquisition of data on macrophyte species composition and abundance, often the only taxonomically verified species lists available and useful for diversity assessments. These data, primarily used to assess the environmental status of macrophyte communities, represent a valuable source for scientific research (long-term data), especially when the scientific community participates/d in the creation of assessment systems. The congress is invited to discuss possible improvements of the monitoring programmes for future scientific use. Results of the first 6 years of annual surveys derived from Baltic Sea and Helgoland macrophyte assessment systems will be presented with regard to their possible contribution to biodiversity research. E.g., the natural part of the Helgoland intertidal area provides different habitats suitable for a diverse algal community of up to 120 species of which about 50-70% can be found during a single survey. Compared to historic records the total number of species remained constant with a shift from brown to green algae. The genus *Fucus* is an important basis of these assessment systems and at the same time an ecologic engineer. The presentation, therefore, will have some special focus on the weal and woe of *Fucus* spp.

9.2 TALK 3 – SYMPOSIA 6 – 11:20–11:40

Desiccation Effects in Terrestrial Streptophycean Green Algae: Transcriptome Analyses of an alpine *Klebsormidum* strain

Burkhard Becker[1], Andreas Holzinger[2]

[1]University of Cologne, Cologne Biocenter, Botany, Köln, Germany
[2]University of Innsbruck, Botanical Institute, Innsbruck, Austria

Green algae are widely recognized as aquatic organisms, however several members of the Streptophyta are terrestrial algae growing e.g. in soil crusts. In the terrestrial habitats these green algae are regularly exposed to high irradiation and desiccation. The physiological and ultrastructural consequences of desiccation have been described recently (Karsten et al. 2010, J Phycol 46: 1187-1197, Holzinger et al. 2011, J Phycol 47: 591-602). To gain more insight the molecular mechanisms of desiccation tolerance in *Klebsormidium* we analyzed the transcriptome of *Klebsormidium crenulatum* (SAG 2415) under moist and desiccated (2.5 h silicagel, RH ~ 10%) conditions using RNAseq. A reference transcriptome (combined samples) was established using the Roche GLS FLX platform (1.5 million reads, 636 million bases, GATC, Konstanz, Germany). The reference transcriptome includes about 24500 sequences. The isolated RNA was analyzed using RNAseq (GATC, Konstanz, Germany). 330 genes are totally suppressed in desiccated samples and 475 genes show less than 10% expression level. Moreover, 32 genes are only found in the desiccated samples and 169 genes are at least 10 fold upregulated. Several upregulated genes show homology to known proteins involved in desiccation tolerance, light and ROS protection.

9.2 TALK 4 – SYMPOSIA 6 – 11:40–12:00

Phosphate transporter of the seagrass *Zostera marina*

Uwe Nehls[1], Stephanie Thomforde[1], Anna Brünner[1]

[1]University of Bremen, Ecology / Botany, Bremen, Germany

Zostera marina is a marine angiosperm flowering plant with a wide distribution in the northern hemisphere. It plays an important role in stabilizing coastline sediments and as nursery ground for fish and other aquatic organisms. In contrast to major competitors (macro algae) *Z. marina* has a root system that is functional in nutrient uptake. Consequently, nutrient uptake from sediments might be of elevated biological impact for seagrasses when nitrogen and phosphate are rare in the water column (mainly in summer). We have therefore identified a number of *Z. marina* phosphate transporter genes and analyzed their expression in plant organs. Three out of a total of nine transporter genes turned out to be preferrently root expressed while the others were expressed in both organs. However, when gene expression was analyzed during the day, one of the preferrently root expressed genes turned out to be temporarily shared between roots and leaves. Furthermore, clear diurnal rhythms were observed for a number of phosphate transporter genes. As expected, transporter gene expression turned out to be strongly influenced by the season. Different functional groups could be distinguished regarding to the time window of their maximal gene expression. Elucidation of phosphate transport properties by heterologous expression in yeast mutants, defective in high affinity phosphate transport, is currently under progress. Our current research state and a functional interpretion of the data are presented.

9.3 TALK 1 – SYMPOSIA 8 – 15:30–16:00

Chemical ecology in the aquatic realm: concepts, approaches and challenges

Eric von Elert[1]

[1]University of Cologne, Zoological Institute, Cologne, Germany

Algae are involved in a suite of biotic interactions such as competition for resources, reproduction and predation by herbivores. Many of these interactions are stirred by chemical signals that do not only mediate these biotic interactions but as well provide a coupling of algal traits to the highly fluctuating natural environment. Such chemical signals comprise pheromones, defensive metabolites, allelopathic compounds, and signals from herbivores. Understanding the role of chemical signaling in these complex interactions with their fluctuations in species composition and fitness over time is challenging, both in planktonic and benthic systems. Examples highlighting several of these interactions will be presented and approaches for the successful identification of the signals involved will be given. Still, conceptual problems arise if the function and evolution of chemical signaling in nature is concerned.

9.3 TALK 2 – SYMPOSIA 8 – 16:00–16:20

Contractile vacuoles (CVs) - an osmoregulatory wonder: continuous water flow through a single celled organism

Karin Komsic-Buchmann[1], Luisa Wöstehoff[1], Burkhard Becker[1]

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The model organism *Chlamydomonas reinhardtii* is a unicellular green alga living in fresh water. The alga possesses a cell wall of hydroxyproline-rich glycoproteins which obviously does not compensate the osmotic water influx. Under hypotonic conditions the cells show two CVs working in osmoregulation. During diastole the CV increases continuously in size followed by a rapid decrease (systole), where the CV expels the collected liquid and fragments into numerous vesicles which fuse with each other and swell to again form the large CV. In this study the cell wall less strain *C. reinhardtii* CC3395 was used. The cells were cultivated under two different culture strategies. Regularly, fresh cultures were inoculated either from cultures of stationary or exponential phase. These cells differ immensely in their CV parameter, their cytosolic osmotic potential and in their plasma membrane's (PM's) osmotic water permeability coefficient Pf. Moreover, the reaction of *C. reinhardtii* to media with different osmotic strength was analyzed. As aquaporins are known to facilitate water flow across membranes, they were analyzed in some detail. Two of the three *Chlamydomonas's* aquaporins are expressed (CreMIP1 & CreMIP3). Both aquaporins were down-regulated under extreme osmotic conditions (hypo- and hyperton). Using GFP as marker, it was found that CreMIP1-GFP is solely localized at the CV membrane.

9.3 TALK 3 – SYMPOSIA 8 – 16:20–16:40

Cellulose degradation and assimilation by the unicellular phototrophic eukaryote *Chlamydomonas reinhardtii*

Lutz Wobbe[1], Olga Blifernez-Klassen[1], Viktor Klassen[1], Anja Doebbe[1], Klaudia Kersting[1], Grimm Philipp[1], Olaf Kruse[1]

[1]Bielefeld University , Biology, Bielefeld, Germany

Plants convert sunlight to biomass, which is primarily composed of lignocellulose, the most abundant natural biopolymer and a potential feedstock for fuel and chemical production. Cellulose assimilation has so far only been described for heterotrophic organisms that rely on photosynthetically active primary producers of organic compounds. Among phototrophs, the unicellular green microalga *Chlamydomonas reinhardtii* is widely known as one of the best established model organisms. It occupies many habitats, including aquatic and soil ecosystems. This ubiquity underscores the versatile metabolic properties of this microorganism. Here we present yet another paradigm of adaptation for *C. reinhardtii*, highlighting its photoheterotrophic ability to utilize cellulose for growth in the absence of other carbon sources. When grown under CO2-limiting conditions in the light, secretion of endo-β-1,4-glucanases by the cell causes digestion of exogenous cellulose, followed by cellobiose uptake and assimilation. Phototrophic microbes like *C. reinhardtii* may thus serve as bio-catalysts for cellulosic biofuel production.

9.3 TALK 4 – SYMPOSIA 8 – 16:40–17:00

How Chlamydomonas Suffers and Recovers from Heat Stress - a Systems Biology Approach

Dorothea Hemme[1,2], Daniel Veyel[2], Timo Mühlhaus[1,2], Frederik Sommer[1,2], Jessica Jüppner[2], Ann-Kathrin Unger[3], Michael Sandmann[4], Ines Fehrle[2], Stephanie Schönfelder[2], Patrick Giavalisco[2], Stefan Geimer[3], Martin Steup[4], Joachim Kopka[2], **Michael Schroda**[1,2]

[1]TU Kaiserslautern, Molecular Biotechnology & Systems biology, Kaiserslautern, Germany
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[3]Universität Bayreuth, Bayreuth, Germany
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To understand how *Chlamydomonas reinhardtii* acclimates to and recovers from thermal stress, we exposed cells to 42°C for 24 h and allowed them to recover at 25°C for 8 h. During this time we monitored protein dynamics via 15N shotgun LC-MS/MS proteomics allowing for the quantification of ~4000 proteins. Furthermore, we analysed levels of ~70 polar (GC-MS) and ~200 lipophilic metabolites (LC-MS). We also monitored cytological parameters and major cellular components like cell size, cell number, cellular ultrastructure (EM), DNA- and protein content. We observed a rapid cell cycle arrest and reduced growth at the onset of heat stress, which came along with a reduced accumulation of ribosomal proteins and a depletion of metabolite pools of the central metabolism. We observed the induction of cellular protection and acclimation mechanisms like an increased accumulation of chaperones, ROS scavengers, compatible solutes and a rapid rearrangement of membrane lipid composition. We observed the accumulation of aberrant structures at regions where multiple thylakoid membranes emerge, presumably arising from disturbed photosystem biogenesis and thus explaining the discrepancy between increased levels of photosystem subunits and decreased oxygen evolution. Surprisingly, not all cellular adaptations to heat stress were reverted when cells started to divide again during recovery.

10 TALK 1 – SYMPOSIA 9 – 10:30–11:00

FROM CLASSICAL INPUT TRAITS TO COMPONENTS OF AN INTEGRATED RESEARCH PORTFOLIO

Jan Dittgen[1]

[1] Bayer CropScience, , Frankfurt

The first part of the talk will focus on classical input traits (herbicide tolerance, insect resistance), their growing impact since their first introduction to the market, the current situation (e.g. development of herbicide resistance), and pipeline projects that are expected to deliver new solutions to the market in the near future. In contrast to these classical monogenetic input traits, complex traits directed to e.g. drought tolerance or yield increase are challenging to realize. Bayer CropScience R&D is aiming to deliver differentiated & sustainable crop solutions including high value seeds and innovative crop protection products based on chemical and biological modes of action. Thus, Breeding and Trait Research are elements of an integrated R&D approach, together with Small Molecules and Biologics. Research in these BCS units will be presented, with special focus on opportunities for young scientists with plant biology background.

10 TALK 2 – SYMPOSIA 9 – 11:00–11:30

Biotech and Breeding for future demands in Agriculture

Katia Schütze[1]

[1] KWS SAAT AG, , Einbeck

KWS is a plant breeding company located in Germany. The core business consists in the development of competitive varieties for several crops (sugar beet, corn, oilseed rape, small grain cereals,sunflower and potato). Breeding, testing and marketing is done on the international level and KWS is active in > 70 countries. Varieties for use as food and feed are now being supplemented by also providing varieties suitable for bio-energy production (bio ethanol and biogas). For the last decade, KWS has actively participated in a number of projects to build up tools, data and expertise in the area of plant genomics and biotechnology. KWS was involved in several projects, "Biologische Baupläne: Entschlüsselung von Resistenzgenen", GABI-BEET, SWEET GABI, GABI Beet Physical Map and as associated partner in the BeetSeq Project. Several ongoings projects run under the Plant 2030 umbrella of the BMBF. This intensive participation in research projects lead to strategies to integrate the results of molecular biology and genomics into the breeding process, mainly by enhanced marker application but also in the identification of candidate genes for transgenic approaches.

10 TALK 3 – SYMPOSIA 9 – 11:30–12:00

Preparing the future : Engineering abiotic stress tolerance in Petunia hybrida

Robert Böhm[1]

[1] Ornamental Bioscience GmbH, Biotechnology, Stuttgart

Enhanced tolerance against adverse environmental conditions (e.g. drought, heat, freeze or salinity) is more and more recognized as future breeding target for crop plants but also in the field of ornamental plants. Ornamental Bioscience GmbH, a joint venture between Selecta Klemm GmbH (Germany) and Mendel Biotechnology (USA) aims to reach this goal by a genetic engineering approach, combining the Mendel transcription factor technology which the genetic pool of Selecta Klemm´s ornamental assortment. Petunia hybrida served as a first model system in which several transcription factors have been introduced by Agrobacterium-mediated gene transfer. After transformation, transgenic lines were subjected to an initial screening for drought and freeze tolerance, as well as to pathogen resistance trials. Promising candidates with enhanced tolerance against one or more of these stress factors were identified. Suitable tests have been designed to characterize the enhanced stress tolerance in these favorite lines in more detail and under different environmental conditions. First promising data and further steps to the market are presented. In the field of ornamental breeding, this is the first commercial genetic engineering approach to improve environmental stress tolerance.

11 PUBLIC EVENING LECTURE

Ancient Pathogen Genomics: Uncovering the evolution and history of historical human and plant pathogens

Johannes Krause[1]

[1]University of Tübingen , Archaeological Sciences, Tübingen, Germany

Genome wide data from ancient microbes may help to understand mechanisms of pathogen evolution and adaptation for emerging and re-emerging human and plant infectious diseases. Using high throughput DNA sequencing in combination with targeted DNA enrichment we have reconstructed medieval bacterial genomes of *Yersinia pestis* and *Mycobacterium leprae* from skeletal remains as well as the causative agent of potato late blight *Phytophtera infestans* from 19th century herbaria collections around the time of the Irish potato famine. We found that the medieval *Y.pestis* strain is ancestral to most extant strains and that the Black Death presents the main historical event responsible for the worldwide dissemination of *Y. pestis*. In contrast the medieval *M. leprae* strains fall within the current genetic diversity of leprosy bacteria. Dating analysis reveal a most recent common ancestor of both all Y.pestis strains and all M.leprae strains within the last 4000 years. The extraordinary preservation of the M.leprae DNA allowed for the first time a *de novo* genome assembly of an ancient organism. The 19th century *P.infestans* genomes derive all from the same clonal lineage HERB-1 that is extinct today. Comparison to modern potato genomes reveal resistancy genes to HERB-1 that are likely a product of historical selective breeding.

ABSTRACTS OF POSTER SESSIONS

POSTER 1 – SESSION 1.1

Functional analysis of RIMB genes in chloroplast-nucleus-signalling and cell vitality

Christiane Hedtmann[1], Heiko Hiltscher[2], Wei Guo[1], Margarete Baier[1]

[1]Freie Universität Berlin, Biology, Chemistry, Pharmacy, Berlin, Germany
[2]HeinHeinrich-Heine-Universität Düsseldorf, Plant Sciences, Düsseldorf, Germany

Reactive oxygen species (ROS) are continuously generated in all aerobic organisms and increase in response to adverse environmental stimuli. In order to prevent the negative influence of ROS on cell functionality, chloroplasts have evolved a highly efficient antioxidant system. We are interested in the functional regulation of antioxidant enzymes in *Arabidopsis thaliana*. All antioxidant enzymes are encoded in the nucleus and posttranslational imported into the plastids. Thus, communication between the spatially separated nucleus and plastid is necessary to regulate the plastid proteome. Signal transduction pathways affecting nuclear transcription of chloroplast antioxidant enzymes were studied in six *Arabidopsis thaliana* redox imbalanced *(rimb)* mutants with decreased promoter activity of the 2-Cysteine peroxiredoxin A (1). Mapping using SSLP marker combined with Illumna sequencing lead to the identification of the mutation in the *rimb1*, *rimb3* and *rimb6* mutant. In the presentation the function of these regulators in chloroplast-to-nucleus signaling and cell vitality regulation will be discussed.

(1) Heiber et al. (2007) Plant Physiology 143: 1774-1788

POSTER 2 – SESSION 1.1

Short-term responses and molecular signalling upon reduced gravitational forces in *Arabidopsis thaliana* cell cultures

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In nature plants are faced with manifold abiotic and biotic signals. Among them, gravity is an ubiquitous factor. Like with any other environmental stimulus, plants have to translate the gravitational signal into a cellular response. To investigate the impact of altered gravitational forces on organisms, a range of experimental platforms is available (space/satellite missions, rocket programmes, parabolic flights, drop tower, ground-based facilities). In order to investigate gravitational signalling upon reduced gravitational forces, we exposed *Arabidopsis thaliana* (cv. Col) cell cultures to parabolic flights on board of an Airbus A300 (Novespace, France). The cells responded to altered g-forces (Mars 0.38g; Moon 0.16g, µg) within seconds. With the onset of µg we measured an increase of cytosolic calcium and H2O2 and a decrease in the NADPH/NADP redox ratio. Responses within the phosphoproteom involved enzymes of primary metabolism [1]. Global transcriptome analysis showed hundreds of differentially expressed genes. E. g. under martian gravity we identified transcripts coding for ROS-detoxifying enzymes (peroxidases), NADPH oxidase, auxin-responsive proteins, transporter and transcriptions factors. The sensitivity to reduced gravity decreased with the number of parabolas (31 parabolas/flight). The data are the basis for a model of gravity sensing.

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[1] Hausmann N., Fengler S. et al. Plant Biology, 2013, in press.

POSTER 3 – SESSION 1.1

Analysis of the high light-responsive AP2 transcription factor AP2-109 in *A. thaliana*

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Chloroplasts must avoid imbalances between photosynthetic metabolism and demand for reducing power and assimilates. To reestablish the metabolic balance e.g. under excess excitation energy, retrograde signaling coordinates chloroplast gene expression in the nucleus with demand. Based on knowledge of stimuli affecting nuclear gene expression, various pathways have been suggested to take part in retrograde signaling, but only few involved elements have been identified. This work deals with the regulation of the AP2/ERF-transcription factor AP2-109 under high light (HL) conditions. AP2 transcription factors have been shown to respond to HL. Bioinformatic analyses of their promoter regions revealed that they share several cis-elements. AP2-109 was chosen as an example for analyzing the promoter activity after transfer to HL. Constructs with shortened promoter-fragments of ERF6 were used to drive expression of ß-glucuronidase (GUS) as reporter in *A. thaliana* protoplasts. After transfection protoplasts were subjected to HL and GUS-activity was quantified in lysates. Changes in GUS activity of the different promoter fragments provide evidence as to which cis-elements are important for the regulation of AP2-109 under HL treatment. The data result in additional clues on upstream regulation of AP2-109 and help to unravel retrograde signals that might initiate the signaling cascade.

POSTER 4 – SESSION 1.1

An enhancer trap study to identify and characterize novel light-responsive *cis*-regulatory elements

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Plants are sessile to their environment and therefore have to cope with adverse conditions, including variable light intensities. Numerous genes are regulated by light, but only little is known about *cis*-regulatory elements which control light acclimation. In order to improve our knowledge of light-regulated gene expression (mainly *cis*-regulation) in plants, *Arabidopsis thaliana* enhancer trap lines were analyzed for the identification of novel light-regulatory *cis*-elements. These lines contain a T-DNA with an open reading frame for *gfp* under the control of the CaMV 35S minimal promoter. The minimal promoter itself is inactive as long as it is not inserted in proximity to an enhancer. Seedlings of *A. thaliana* enhancer trap lines were grown under various light intensities and light quality regimes. Selected lines showed a correlation between light condition and GFP expression. The identification of the T-DNA insertion sites leads to candidates for novel light-responsive *cis*-regulatory elements. These candidates are further investigated by using real time polymerase chain reactions (qRT-PCR). Additionally, the putative promoters were analyzed by construction of several different promoter reporter gene constructs in consideration of a previous *in-silico* analysis. Initial results of the promoter studies will be presented.

POSTER 5 – SESSION 1.1

The redox-dependent switch of the chloroplast 2-cysteine peroxiredoxin between peroxidase and chaperone and its possible involvement in cell signalling

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In plants, the highly abundant 2-Cysteine peroxiredoxin (2-CysPrx) is involved in protecting photosynthesis and plays an essential role in antioxidant defense and H2O2-mediated signaling. In dependence on its redox state, the chloroplast 2-CysPrx switches between its function as peroxidase and chaperone. This study aimed at linking functional diversity of 2-CysPrx to conformation. The transition from low to high molecular weight oligomers depends on the redox state of the conserved catalytic Cys. Therefore, different site-directed mutagenized 2-CysPrx variants were engineered. They are fixed in naturally occurring conformations and functions, namely reduced (C54S and C176S), hyperoxidised (C54D), oxidized (C54DC176K) and phosphorylated form (T92D), or reveal reduced aggregation capacity (F84R). A carboxyterminally truncated form has decreased hyperoxidation propensity (ΔC (-20 aa)). The highest peroxidatic activity was seen for the variants F84R and ΔC while the hyperoxidised C54D showed the highest chaperone activity. Analysis of the interaction of the variants with NADPH thioredoxin reductase C (NTRC) suggests that the interaction depends on the cysteine residues but especially the C-terminal tail. The results indicate that the flexibility of the protein structure is essential for the evolutionarily conserved switch between peroxidase and chaperone function.

POSTER 6 – SESSION 1.1

A. thaliana Glutaredoxin S17 (AtGRXS17) is under longday conditions involved in several developmental processes

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Glutaredoxins and thioredoxins belong to the large protein family of redoxins. GRXS17 consists of a thioredoxin (Trx) and between one and three glutaredoxin (Grx) homology domains (HD). It has been identified, with a similar domain composition in all eukaryotic organisms characterized to date, carrying different names. The composition of one Trx-HD and three Grx-HD is only found in higher plants. A function link, connecting GrxS17 from *A. thaliana* (AtGRXS17) with transcription factors and kinases, was identified by *in vitro* protein interaction studies, suggesting a role in developmental processes such as flowering induction and/or flower organ formation. Bimolecular Fluorescence Complementation (BiFC) verified the interactions of GrxS17 with the kinase At1g50570 and the CCAAT-transcription factor NF-YC11 (At3g12480), confirming the interaction identified by mass spectrometric analysis of pull-down experiments. The three Grx-HD of the AtGRXS17-protein were found to coordinate [2Fe-2S]-clusters. We hypothesize that AtGRXS17 may regulate transcription in a [2Fe-2S]-cluster dependent manner. This hypothesis is supported by analysis of AtGRXS17 T-DNA-insertion mutants, which generate various developmental phenotypes under long-day conditions (LD), while under short-day conditions (SD) the plants do not exhibit any developmental deviations compared to WT plants.

POSTER 7 – SESSION 1.1

S-glutathionylation of *A. thaliana* peroxiredoxin II E

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Peroxiredoxins (Prx-s) constitute a family of thiol-dependent peroxidases that are part of the enzymatic antioxidant defence system, but also participate in cell signaling. Prx-s detoxify a broad range of hydroperoxide substrates. Multiple functions have been assigned to the various members of this protein family, including activity as peroxidase, chaperone and thiol oxidase, or as enzyme activator and redox sensor. Plastids are sites of rapid production of reactive oxygen species (ROS) in photosynthesis and other metabolic pathways. Three types of Prx-s, namely 2-CysPrx, Prx Q and type II peroxiredoxin E (PrxIIE; At3g52960) reside in the plastids. Here we present data on the activity and regulation of chloroplast PrxIIE. By mass spectrometric analysis and immunoreaction it will be shown that PrxIIE is subjected to S-glutathionylation of its peroxidatic Cys121. Glutathionylation occurs if the proportion of oxidized glutathione (GSSG) in the total glutathione pool increases within physiological ranges. Glutathionylation is accompanied by inhibition of peroxidase activity. Deglutathionylation via glutaredoxins (Grxs) and sulfiredoxins (Srx) was investigated in detail. The results point to a redox-dependent mechanism by reversible glutathionylation which controls the peroxidase and signaling functions of PrxIIE.

POSTER 8 – SESSION 1.1

Identification of ACTIVE PHYA BINDING 1 (AAB1) - A novel protein interacting with the Pfr form of PHYTOCHROME A

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Plants being sessile organisms rely on the ability to detect light in a qualitative and quantitative manner. For this purpose they possess several classes of photoreceptors. The phytochromes (PHYs) perceive light in the red and far-red light range of the visible spectrum. In *Arabidopsis thaliana*, the PHY family comprises five members (PHYA-E), PHYA and PHYB being the most important PHYs for plant development. PHYA is essential for far-red light perception and contributes to deetiolation in strong canopy shade and the regulation of flowering. It is rapidly transported into the nucleus upon activation by light using a PHYA-specific transport mechanism. In contrast to PHYB-E, PHYA is photolabile and quickly degraded in its active form, this being an intrinsic property of the PHYA receptor-signalling pathway. In a Y2H screen for interaction partners that bind to the light-activated form of PHYA, we identified a novel protein termed AAB1 (for ACTIVE PHYA BINDING PROTEIN 1). This protein preferentially interacts with Pfr, whereas no binding to the inactive form of PHYA, Pr, is detectable. Localisation studies in transiently transformed tobacco further revealed a co-localisation of AAB1 in PHYA dependant nuclear bodies, suggesting that AAB1 may be involved in either PHYA signalling or degradation. Analysis of an aab1 T-DNA insertion mutant supports a role in PHYA mediated control of flowering time.

POSTER 9 – SESSION 1.1

Fast retrograde signaling in respond to high light is mediated by metabolite export, MAP kinase 6 and an AP2/ERF transcription factor co-expression network

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The molecular acclimation towards changing environmental conditions is crucial for plants to sustain optimal growth and to balance defense responses with maximal reproductive fitness. Especially with rapidly changing parameters like light and temperature, an immediate regulation of photosynthesis is needed to reestablish metabolic homeostasis and to avoid damage development. In this context retrograde signaling from the chloroplast to the nucleus is important to transmit information on the metabolic state of the chloroplast to nuclear gene expression. A co-expression network of AP2/ERF transcription factors (TF) was identified in *Arabidopsis thaliana* and found to rapidly respond within 10 min after transfer of low light acclimated plants to high light. Initiation of the transcriptional response was completed within 1 min after transfer. The fast expressional response of the four AP2/ERF TFs 103, 109, 113 and 115 was deregulated in triosephosphate/phosphate translocator (*tpt*) mutants. Activation of mitogen activated protein kinase 6 (MPK6) after 1 min was absent in the *tpt* mutant as compared to wild type. Together with altered transcript regulation in *mpk6* and *erf109* mutants this suggests a novel retrograde signaling pathway which mediates high light acclimation independent on previously described redox and hormonal pathways.

POSTER 10 – SESSION 1.2

Growth control by phytosulfokine signaling

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Phytosulfokine (PSK) is a peptide growth factor that is characterized by two sulfated tyrosine residues. The PSK ligand is perceived by leucine-rich repeat receptor kinases. Arabidopsis has two genes that encode the PSK receptors PSKR1 and PSKR2. PSK signaling mainly through PSKR1 was shown to control cell expansion whereas PSKR2 plays a minor role. Knockout of both PSK receptors results in shorter roots and shorter hypocotyls in Arabidopsis seedlings. PSK signaling in the epidermis is crucial for seedling growth as was shown by tissue-type specific expression of PSKR1 in the *pskr1-3 pskr2-1* double knockout background. The contribution of receptor kinase activity has not been studied to date. Nor have downstream signaling events been described. Our research is aimed to analyze receptor activity in relation to growth control through mutational analysis of conserved residues of the receptor protein. *In vitro* phosphorylation assays of wildtype and mutated receptor protein are combined with *in planta* analysis of the growth mediated by mutated receptor protein.

POSTER 11 – SESSION 1.2

HORMONAL TANGO: HOW AUXIN AND BRASSINOSTEROIDS INTERACTIONS SHAPE PLANT DEVELOPMENT

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Multiple mechanisms have been described for coordination of responses to the plant hormones auxin and brassinosteroids. One unexplained phenomenon is the reliance of the auxin transcriptional response on a functional brassinosteroid pathway. Previous research has demonstrated that a bipartite element, containing a Hormone Up at Dawn (HUD)-type E-box and an AuxRE-related element, were required for auxin and brassinosteroids sensitivity. The transcription factors BRASSINOSTEROID INSENSITIVE1-EMS SUPPESSOR1 (BES1) and MONOPTEROS (MP)/ AUXIN RESPONSE FACTOR5 (ARF5) show enhanced binding to this element. To identify shared targets of MP/ARF5 and BES1, we have searched the *Arabidopsis* genome for promoters containing bipartite regulatory elements. Several of the genes identified in our bioinformatic analysis respond to both auxin and brassinosteroid application, and their promoters show evidence of binding by both BES1 and MP/ARF5. Moreover, we found that MP/ARF5 binding could be enhanced in *bes1-D* mutant plants where BES1 is strongly stabilized. Altogether, these data suggest that BES1 binding enhances MP/ARF5 activation of a specific subset of target genes. Functional relevance of this dual-control module will be discussed.

POSTER 12 – Session 1.2

The cytokinin receptors AHK2 and AHK3 play a central role in the etioplast - chloroplast transition

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A role of the plant hormone cytokinin in regulating the development and activity of chloroplasts was described soon after its discovery. However, most observations reporting this cytokinin function were descriptive and the molecular mechanisms underlying this activity remain elusive despite great progress in understanding the metabolism and cellular signalling of the hormone. The development of chloroplasts from etioplasts is an important event during the dark-to-light transition of seedlings. It is marked by ultrastructural changes of the plastids and coincides with the biosynthesis of chlorophyll. Etiolated seedlings of *Arabidopsis thaliana* which were mutated in two of the three cytokinin receptor genes (*ahk2 ahk3* mutants) showed a reduced greening rate in comparison to wild-type seedlings. This indicated a central role for the cytokinin receptors AHK2 and AHK3 in the greening response. We show that this is due to a strongly reduced and delayed light-responsiveness of the rate-limiting steps of 5-aminolaevulinic acid formation and of the first enzymatic steps of the chlorophyll branch during tetrapyrrole biosynthesis. These results demonstrate that a fully functional cytokinin perception is essential for normal chloroplast development during dark-to-light transition and open the path to obtain a more detailed understanding of this classical cytokinin function.

POSTER 13 – SESSION 1.2

Dynamics of Auxin Sensing by SCFTIR1/AFB-AUX/IAA Co-Receptor Complexes in Arabidopsis

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Auxin is a key regulator of plant growth and development. It is perceived by a co-receptor system formed by a TIR1/AFB F-box protein and an AUX/IAA transcriptional repressor. TIR1/AFBs act as substrate receptors of SCF-type E3 ubiquitin ligases, recognizing the degron of AUX/IAAs and targeting them to the proteasome. So, derepression of auxin response genes takes place. The diversity of AUX/IAA, and TIR1/AFB protein families gives rise to numerous possible co-receptor combinations. We hypothesize that plant cells perceive different auxins and auxin concentrations via assembly of co-receptors with distinct sensing properties. Here, we are interested in the biochemical features and structure-function relationship of auxin co-receptors, and the specific downstream events they trigger. We have demonstrated the assembly and auxin-binding capability of selected co-receptors *in vitro*, and have shown differential auxin-dependent TIR1/AFB-AUX/IAA interactions in yeast. Currently, we are studying regions outside the conserved degron that seem to contribute to auxin receptor complex formation. We also have ongoing experiments to track AUX/IAA stability in various genetic backgrounds, in order to correlate biochemical properties of co-receptors with physiological responses *in planta*. Our studies will contribute to understanding small molecule perception and its downstream events.

POSTER 14 – Session 1.2

A green/red synthetic biology approach towards the development of a quantitative ratiometric sensor for time-resolved analysis of auxin dynamics

Sabrina Wend[1], Cristina Dal Bosco[1], Klaus Palme[1,2], Wilfried Weber[1,2], Alexander Dovzhenko[1], **Matias Zurbriggen**[1,2]

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Time-resolved quantitative analysis of auxin-mediated processes in plant cells is as of yet limited. By applying a synergistic mammalian and plant synthetic biology approach, we have developed a novel ratiometric luminescent biosensor with wide applicability in the study of auxin metabolism, transport, and signalling in single cell systems, as shown here for selected proof of principle applications. The sensor enabled quantitative time-resolved monitoring of intracellular auxin changes upon exogenous application of natural and synthetic auxins (chemical assay prototype) or carrier-mediated modulation of auxin levels in cells (genetic assay prototype). The suitability of the genetically encoded biosensor for rapid, robust and highly sensitive auxin analysis open new perspectives for the analysis of complex auxin dynamics in plant growth and development.

POSTER 15 – Session 1.2

Structure-function studies on the PsIAA4 dimerization domain provide insights into auxin-dependent transcriptional regulation

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Auxin responses depend on gene expression regulated by AUXIN/INDOLE-3-ACETIC ACID INDUCIBLE (AUX/IAAs) and AUXIN RESPONSE FACTORS (ARFs) transcription factors. *AUX/IAA* genes are rapidly induced by auxin and encode short-lived nuclear proteins with four conserved domains (DI-IV). While DI recruits transcriptional co-repressors, DII acts as a degron, which together with TIR1/AFBs constitutes a minimal auxin co-receptor complex. C-terminal domains III-IV (CTD) mediate homo- and hetero-dimerization between AUX/IAAs and ARFs. Previously, DIII was predicted to adopt a βαα-fold similar to a prokaryotic transcription factor family. However, based on our high resolution NMR structure of the PsIAA4 CTD we could demonstrate that DIII-IV adopts a globular ubiquitin-like/β-grasp fold resembling the Phox and Bem1p (PB1) protein-protein interaction domains. Those interact with each other in a head-to-tail fashion via salt bridges between conserved acidic and basic patches. Mutational studies in yeast showed that double mutations in those patches abolish PsIAA4 homo-dimeric interactions. Currently, we are performing *in vitro* and *in silico* mutational studies of PsIAA4 CTD to map interface residues more precisely. Our structural studies on AUX/IAAs will contribute to the understanding of how auxin-dependent transcription and gene activation work in plants.

POSTER 16 – Session 1.2

Analysis of new genetically engineered AUXIN-BINDING-PROTEIN 1 (ABP1) mutants reveal their functions in auxin and red light physiology

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We isolated four stable abp1 mutants expressing point-mutated or wt ABP1 cDNA in wt-ABP1-null background. WE found all auxin functions were of partial loss-of function in the mutants: root and hypocotyl gravitropism, hypocotyl phototropism, main root growth control and lateral root formation, basipetal auxin transport in root tips, lobe extension in leaf epidermis cells, and apical dominance. Expression of most early auxin-induced genes (10 µM 1-NAA) was delayed as soon as after 10 min in the mutants. In tir1 only 2 genes were delayed in expression after 30 min. Unexpectedly, red light-dependent responses were also diminished in abp1 mutants. Hypocotyls were already taller in white light, R- and FR- insensitive, and showed hypersensitive elongation in shade. tir1 did not respond to shade conditions. A blue-insensitive hypocotyl response was not found. abp1 mutants flowered early, leaves were broader and longer and had a larger blade area. FR-induced genes (after 1h) were induced less and repression of these genes by R was lowered. We suggest that ABP1 has a capacity to regulate auxin transport and thus influences TIR1-dependent gene expression (Effendi et al. Plant J. 65, 282-294). The phyB-like part of the phenotype is hypothetically explained as co-regulation of auxin- and light-dependent genes.

POSTER 17 – SESSION 1.2

14-3-3 proteins meet auxin transport

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14-3-3 proteins are well known to bind diverse target proteins in a phosphorylation-dependent manner, thereby modifying the activity state of their clients. We used ethanol-inducible RNAi /amiRNA to suppress the function of three 14-3-3 isoforms belonging to the ancestral epsilon group. Remarkably, EtOH treatment reveals severely affected developmental processes that are frequently observed in auxin mutants. Indeed, the underlying cause of the phenotype is a significantly reduced capability to transport auxin, which finally results in an elevated auxin level in aerial tissue and a reduced auxin level in roots. So, what might be the molecular basis of 14-3-3 dependent regulation of auxin transport? The activity of the plasma membrane H+-ATPase is crucial for polar auxin transport and furthermore, this H+-pump is activated by association of 14-3-3s. However, we neither could phenocopy the RNAi phenotype in WT by pharmacological inhibition of the pump nor rescue the phenotype by expression of a constitutively active H+-ATPase. We therefore focused on the analysis of PIN auxin efflux carriers. Remarkably, ethanol induction results in a significant increase in the amount of PIN proteins while the transcript level is unaltered. This stabilization might be due to altered membrane trafficking.

POSTER 18 – SESSION 1.2

The type-B response regulator ARR18 mediates gene expression through direct interaction with a bZIP63 transcription factor

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In *Arabidopsis*, two-component system (TCS) involves three different types of proteins namely the histidine kinases (AHKs), the histidine phosphotransfer proteins (AHPs) and the response regulators (ARRs). The histidine kinase AHK1 has been proposed to function as an osmosensor. However, little information is available concerning AHK1 downstream signaling. Here we show that the B-type response regulator ARR18 functions as a positive osmotic stress regulator in *Arabidopsis* seeds, potentially acting downstream of AHK1. Furthermore, it affects the activity of seed-specific promoters known to be controlled by basic leucine zipper (bZIP) transcription factors. The modulation of the promoter activity appears to result from the direct interaction of the response regulator with the bZIP protein. We unravel a phosphorylation-dependent interaction of ARR18 with bZIP63. Utilizing *Arabidopsis* derived protoplasts in reporter gene assays, we show that ARR18 interaction negatively interferes with the transcriptional activity of bZIP63. Moreover, overexpression of bZIP63 causes enhanced sensitivity of *Arabidopsis* seeds to osmotic stress. Thus, our studies provide new insight into the function of ARR18 and bZIP63 as antagonistic regulators in osmotic stress signaling in *Arabidopsis thaliana*, demonstrating at mechanistic level how the TCS is able to modulate bZIP-dependent responses.

POSTER 19 – Session 1.2

ARR1 and ARR2 are involved in flowering time regulation in *Arabidopsis thaliana*

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The Arabidopsis response regulators 1 and 2 (ARR1, ARR2), canonical members of B-type response regulators in the two-component system (TCS) phosphorelay circuit serve as molecular hubs integrating several incoming signals. Detailed analysis of *arr1* and *arr2* single loss-of-function mutants and the double mutant revealed an early flowering phenotype under short day conditions (SD) with the *arr1arr2* double mutant having an additive effect. Under flowering-inductive long day (LD) conditions, mutants again showed similar phenotype but with weaker penetrance. Despite similar sequence homology of the *ARR1/ARR2*, the loss of *ARR2* effects the flowering time more strongly than *ARR1*. The lack of *ARR1/ARR2* activity causes down-regulation of specific floral repressors but not of floral activators. *ARR2* expressed either in the shoot apical meristem (SAM) or in phloem companion cells recues the early flowering phenotype of the *arr2* and the *arr1arr2*. The crossing of *ARR1/ARR2* mutants with flowering time-related mutants revealed that *ARR1/ARR2* function predominantly independent of *Flowering Locus C* (*FLC*) and *Flowering Locus M* (*FLM*) acts epistatic to *ARR1* and *ARR2*. *ARR2* also functions mainly upstream of *Suppressor of overexpression of constans1* (SOC1) and *Constans* (*CO*). The functional implications of the involvement of ARR1 and ARR2 and, thus, the TCS in flowering time control will be discussed.

POSTER 20 – SESSION 1.3

Proteomic changes of Suaeda maritima observed under hypoxic conditions in relation to its physiological basis

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The potential of specific traits of a genotype to respond to different environmental conditions, is an important adaptive mechanism for minimising potentially adverse effects of environmental fluctuations in space and time. *Suaeda maritima* shows morphologically different forms on high and low areas of the same salt-marsh. Our aims were to examine whether these phenotypic differences occurred as a result of plastic responses to the environment. Soil redox state, indicative of oxygen supply was examined as a factor causing the observed morphological and protein biochemical differences. High LDH activity in roots of plants grown in aeration and in hypoxia indicated pre-adaptation to fluctuating root aeration and could be a factor in the phenotypic plasticity and growth of *S. maritima* over the full tidal range of the salt-marsh environment. Twenty six proteins were up-regulated under hypoxic conditions. Plasticity of morphological traits for growth form at extremes of the soil oxygenation spectrum of the tidal salt-marsh did not correlate with the physiological plasticity of constitutively high lactate dehydrogenase found in the roots.

Wetson A. M. Zörb C., John E. A., Flowers T. J. (2012) High phenotypic plasticity of Suaeda maritima observed under hypoxic conditions in relation to its physiological basis. Annals of Botany 109: 1027-1036.

POSTER 21 – SESSION 1.3

„The chloroplast antioxidant system in priming stress responses - short cold challenges for inducing cold memory"

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For most plants cold occurs regularly in natural environments. In most cases longer cold phases are preceded by a short and transient decrease in temperature. We propose that plants get primed during this first cold phase in response to redox signals. Upon cold stress increasing amounts of reactive oxygen species are formed due to a reduction in electron consumption by the Calvin cycle [1], changes in enzyme activity and membrane fluidity [2]. Redox imbalances stimulate the expression of chloroplast antioxidant enzymes. Subsequently the composition of the chloroplast antioxidant system controls redox signals. We hypothesize that this altered setting acts as stress memory. qPCR experiments show that cold orders the beforehand noisy transcription of antioxidant genes, *e.g.* stromal ascorbate peroxidase (sAPX). To test whether a previous received cold stimulus alters the answer to the second, we challenged four *A. thaliana* accessions twice for 24h with 4°C. Three accessions showed differences in transcription level directly after the first and second challenge for sAPX and thylakoid ascorbate peroxidase (tAPX). Transcript abundance regulation indicates a specific function of sAPX and tAPX regulation in overall cold-response strategy of Arabidopsis.

[1] Maruta T et al. 2012, Journal of Biological Chemistry 2012 Apr; 287

[2] Vickers CE et al. 2009, nature chemical biology 2009 May; 5

POSTER 22 – Session 1.3

The chloroplast antioxidant system and developing strategic responses to long cold priming stress in *Arabidopsis thaliana* ecotypes

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For sessile organisms, like higher plants, difficulties in development and distribution are caused by various environmental factors of which extreme temperatures have a strong impact. During cold-induced destabilization numerous physiological and molecular changes occur. Recent studies showed that long-term exposure to low temperatures causes changes in energy absorption level and photochemical transformation. As a result, carbohydrate metabolism is reorganized and the antioxidant capacity increase.

We consider the hypothesis that the transformation of the chloroplast antioxidant system acts as a medium term memory which is involved in priming plants and modulating the sensitivity to a second cold stimulus. Special interests are on memory functions and molecular study of storing and recalling information. The focuses are on analyzing the long cold priming (14 days, 4°C) data and there effect on triggering (1 day, 4°C) by qRT-PCR, determination the costs and benefits of priming via fitness data and observation of memory effects on long priming by performing analysis of metabolites. In the first data set the impact of long-term cold on the chloroplast antioxidant system were quantified. The responses to the triggering stimulus have been recorded. It will be shown that investment into priming brings accession-specific benefits.

POSTER 23 – SESSION 1.3

Investigation of the transcription factor network in the regulation of iron homeostasis in *Arabidopsis thaliana*.

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Iron is a very important micronutrient for most organisms due to its reduction-oxidation property. Iron mobilization and uptake in plant cells are regulated by a network of transcription factors (TFs) such as the four basic Helix-Loop-Helix (bHLH) TFs from the subgroup Ib(2)-bHLH038, bHLH039, bHLH100 and bHLH101, which act together with the major iron-uptake regulator- FIT. The exact functions of these four bHLHs have been unclear until now due to their partial functional redundancy. We have focused our attention on the functional interaction between FIT and bHLH039 and its effect on the regulation of iron uptake and mobilization. Microarray analysis on wild type and HA3-bHLH039 over-expression plants (39Ox) grown at iron deficiency and sufficiency yielded about 900 differential regulated genes. In order to investigate the influence of bHLH039 over-expression on FIT function and iron-uptake regulation, we crossed 39Ox plants with *fit,* FIT-GFP and pFIT::GUS. This allows us to draw conclusions on the indispensability of FIT for the activation of the iron-uptake machinery. Furthermore, an antiserum against bHLH039 was generated with the aim to monitor the response of endogenous bHLH039 protein levels to different iron supply or FIT functionality.

POSTER 24 – Session 1.3

CDPKs in priming in response to low temperature in *Arabidopsis thaliana*

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CDPKs are calcium-binding protein kinases which were shown to play important roles in early signal transduction pathways in abiotic and biotic stress responses. Also one of the first responses to cold stress is the release of calcium ions into the cytosol with stress specific oszillation, which can be decoded by CDPKs. Cold stress leads to diverse changes in metabolism and morphology of the whole plant and reprogramming of around 14% of all genes in *Arabidopsis thaliana*. By an exposure to mild chilling stress (4°C) plants can be primed to a later cold stress of subzero degree (triggering stimulus). CDPKs may be involved in the onset and maintenance of this cold priming and cold memory. We were able to identify different CDPKs which show a differential expression during cold stress. Aim of this project is the detailed characterisation of this candidate AtCPK isoforms involved in cold priming process and the elucidation of the molecular mechanism of cold priming mediated by the kinases. Wild-type plants but also mutant and transgenic CDPK lines will be analyzed by using qRTPCR, metabolite analyses and biochemical approaches.

POSTER 25 – SESSION 1.4

Functional characterization of Rice Cytochrome-P450 *Os08g01480* in *Arabidopsis* suggests its role in different abiotic stress

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Heavy metals get concentrated in soil leading to its mobilization in different plant parts and dietary consumption of plants also shows adverse influence on human health. Many physiological activities of plants get affected by heavy metal stress. Cytochrome P450s (P450s) composed of extensive classes of enzymes and plays an important role in many fundamental pathways including detoxification of exogenous toxic molecules both in animals and plants (xenobiotics). In the present study we cloned one CytP450 (*Os08g01480*) which was up-regulated during arsenic stress in rice and overexpressed it in *Arabidopsis*. Homozygous overexpressing lines shows its role in different abiotic stress. To investigate the regulation of *Os08g01480* gene expression, the promoter of rice *Os08g01480* gene, was fused to a *GUS* reporter gene, and the recombinant transgene was introduced into *Arabidopsis*. *Os08g01480* gene has the highest promoter activity in reproductive parts. We also analyzed the promoter activity under different heavy metal stress and other abiotic stresses. The Cytochrome P450 belongs to a multi-gene family and this gene has a unique expression profile during growth, development and different stress conditions. All these studies indicate that the expression of cytochrome P450 is differentially modulated during different environmental stress as well as in different developmental stages.

POSTER 26 – SESSION 1.4

Ceratophyllum as a model for analysing toxicity and detoxification of cadmium in plant shoots

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We used the aquatic model plant *Ceratophyllum demersum* to study toxicity and detoxification of Cd under environmentally relevant conditions. High, moderate and low concentrations of Cd had different effects. Lethally toxic concentrations (100-200nM Cd2+) led to growth stop and the plant's ability to perform photosynthesis (measured as Fv/Fm) decreased more than twofold, consistent with decreased pigment content. Moderately toxic concentrations (10-50nM Cd) led to reduced growth, slightly reduced pigment content, but hardly affected photosynthesis (measured as O2 exchange and as Fv/Fm). Lower concentrations (0.2-5nM Cd) even had beneficial effects, like enhanced growth rate. When applied in low concentrations, Cd was homogeneously distributed in the whole cross section of the leaves like a nutrient. Moderate and high Cd concentrations led to sequestration of Cd in the vascular bundle and the epidermis cells, where Cd does not affect photosynthetic molecules. At toxic Cd concentrations, Zn was redistributed and mainly found in the vein along with Cd, indicating inhibition of Zn transporters. Consistently, we found that the induction of phytochelatins is not proportional to metal concentration, but has distinct thresholds, specific for each PC species. PC3 especially was switch-like induced already at 20 nM Cd, which was previously regarded as non-toxic to most plants.

POSTER 27 – Session 1.4

Evolution of *Cis*-Regulatory Elements of *Heavy Metal ATPase4* Promoter in Zn/Cd Hyperaccumulator *Arabidopsis halleri*

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The heavy metal hyperaccumulator *Arabidopsis halleri*, an extremophile sister species of *Arabidopsis thaliana* is a powerful model for studies on plant adaptation and phytoremediation. *Heavy Metal ATPase4 (HMA4)* transcript levels are strongly elevated in *A. halleri* relative to *A. thaliana* due to *cis*-regulatory changes and a tandem triplication of the gene. Our objective is now to identify the *cis*-regulatory elements that govern the differences in *HMA4* promoter activity between *A. halleri* and *A. thaliana*. Using *GUS* reporter gene-based histochemical and fluorometric analysis, we examined promoter activity in a series of *AtHMA4* and *AhHMA4-1* deletion constructs in transformants of *A. thaliana*. Fragments between 2,326 bp and 130 bp upstream the transcriptional start site of *AhHMA4-1* promoter resulted in *GUS* activity similar to when the wild type *AhHMA4-1* promoter is used. Similarly, fragments between 2,799 bp and 129 bp of *AtHMA4* resulted in activity approximately two orders of magnitude lower than the *A. halleri* fragments, and similar to wild type *AtHMA4*. This suggests that the regions deleted so far do not contain *cis*-regulatory element(s) for the transcriptional regulation of *AtHMA4* and *AhHMA4-1*. A pair-wise comparison of the 5'UTR and 130/129 bp regions between *AtHMA4* and *AhHMA4-1* promoter showed low mutual sequence identity. We speculate that these sequence differences may include the presence/absence of one or more *cis*-regulatory element(s) responsible for the regulation of *AtHMA4* or *AhHMA4-1*. Further work is in progress.

POSTER 28 – SESSION 1.4

Biochemical and physiological evidence characterising chromium as a new essential ultra-micronutrient in plants

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Chromium is considered as non-essential for plants, and the activation of insulin that was suggested as the role of Cr in animals does not exist in plants. Using conditions that allowed work in the sub-nM range, we found that the water plant *Ceratophyllum demersum* stopped growth unless Cr(III) as Cr3+ or Cr(VI) as CrO42- became available, as extrapolated from the growth decrease towards the lowest achievable Cr (0.17 nM). Cr deficiency was furthermore inducible in *Glycine soja* (soybean), and to a lesser extent in wheat (*Triticum aestivum*). In all species Cr deficiency led to retarded formation of new meristems, in soybean sometimes to death of meristems. Investigating Cr deficiency effects further in *C. demersum* as a plant shoot model revealed that non-photochemical exciton quenching and photosynthetic oxygen release were affected by deficient Cr. Metalloproteomics applying radioactive 51Cr, native gel electrophoresis and size exclusion chromatography with element-sensitive detection in extracts from plants and cyanobacteria showed Cr in at least one >50 kDa membrane protein plus several <6 kDa soluble proteins. The large membrane protein did not bind the chemically similar Mo, and also element uptake did not suggest a replacement of Mo by Cr. All these results suggest that Cr is an essential ultra-micronutrient in plants, meaning that Cr has functions other than insulin activation. Further characterisation and identification of the Cr-binding proteins is currently in progress.

POSTER 29 – SESSION 1.4

Phosphate sensing in root meristems: Functional interaction of PDR2 and LPR1/LPR2

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Phosphate (Pi) is the second most limiting macronutrient. To maintain Pi homeostasis, Arabidopsis plants deploy systemic and local Pi deficiency responses to adjust central metabolism (Pi economy) and root development (Pi acquisition), respectively. Pi shortage stimulates formation of a shallow root system by attenuating primary root extension and promoting lateral root formation. We previously characterized a set of mutants with opposite primary root growth phenotypes on low Pi, the hypersensitive (short root) *pdr2* (*Pi-deficiency response2*) and insensitive (long root) *lpr1* and *lpr2* (*low Pi root*) lines. We showed that the ER-localized PDR2 P5-type ATPase and LPR multicopper oxidases functionally interact in a Pi-sensitive pathway to adjust root meristem activity via Fe redistribution and cell type-specific callose deposition in the stem cell niche. Our genetic analysis suggests PDR2-dependent restriction of LPR output. To support the model, we generated LPR1 and LPR2 overexpression lines, which display a *pdr2*-like primary root phenotype, Fe accumulation, and callose deposition in low Pi condition only. We are studying how PDR2 regulates LPR output, either by directly regulating LPR biogenesis/function, or by interfering with products of LPR MCO activity. We propose that LPRs function as ferroxidases to specifically modulate Fe homeostasis in root meristems during Pi shortage.

POSTER 30 – SESSION 1.5

Analysis on the Diversity of Drought Tolerance in Grasses of the genus *Panicum*

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Due to the predicted climate change, increasingly severe droughts and temperatures will have a great impact on biodiversity. Plants using C4 photosynthesis have a competitive advantage over those using the C3 photosynthetic pathway because of lower photorespiration and a threefold higher water use efficiency. Additionally, the optimal growth temperature for C4 plants ranges from 30-45 °C while that for C3 plants is about 15-25 °C. These parameters favour C4 grasses in the West African Savannah. Within the genus *Panicum* the C3 type and all C4 subtype of photosynthesis are realized. Members of this genus not only dominate the landscape in the West African Savannah but also serve as crop and pasture grasses. To ascertain the cause for differences in the ability to adapt to drought stress, three species of the genus *Panicum* were analysed by proteomic and transcriptomic approaches under control, drought and recovery conditions. *P. turgidum* is known to be extremely drought tolerant, *P. laetum* as well as *P. miliaceum* (all C4 NAD-ME) are less drought tolerant and *P. bisulcatum* (C3) serves as a control being sensitive to drought. By comparing these three species, differences in gene expression analysed by HT-SuperSAGE, protein profiles (Western Blots) and physiological adaptations can help understanding the mechanisms of drought adaptation and resistance.

POSTER 31 – SESSION 1.5

GABA is involved in stomatal movement

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The four-carbon non-proteinogenic amino acid gamma-aminobutyric acid (GABA) is produced from glutamate by the action of the GABA-forming enzyme glutamate decarboxylase (GAD) in the cytosol. GABA is known to increase in the plant's response to several abiotic stresses, among them drought and salt stress. By impairing GAD activity, we created GABA-defective plants that were unable to respond to drought and salt stress in an adequate manner, i.e., they displayed an early wilting phenotype upon stress application. The plants were found to have a constitutively increased transpiration rate leading to early wilting when drought or salt stress is applied. Possible roles of GABA in stomatal movement will be discussed.

POSTER 32 – SESSION 1.5

Improving crop stress tolerance by single and combinatorial genetic transformation and multiple gene stacking with selected members of the *CDPK* gene family.

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Calcium is a core transducer and regulator of abiotic and biotic stress signals. Recent research has demonstrated that in stress signaling several essential steps are influenced by Calcium-dependent protein kinases (CDPK) such as perception, transduction and gene expression regulation finally resulting in the appropriate stress responses. This was achieved by mutant analysis and/or single overexpression of *CDPK*s or of truncated (VK) and kinase deficient variants (mut) in Arabidopsis. The overexpression of *CDPK*s and their respective variants demonstrated a clear increase in stress tolerance dependent on kinase function, particular under severe drought as well as cold stress conditions. Results obtained in *Arabidopsis thaliana* could be confirmed in tobacco plants overexpressing the same *CDPK*. Molecular analysis highlighted that the tolerance is mediated via established pathways such as the accumulation of osmotic active compounds like proline in response to cold. Selected members of the Arabidopsis *CDPK* gene family were used for combinatorial transformation in tobacco and the resulting lines will be screened for enhanced tolerance addressing the question if multiple gene stacking improving tolerance even more compared to the existing tolerant single gene overexpression lines.

The project was supported by the Croptimise Consortium [0315959A] in frame of the BMBF 2030 initiative.

POSTER 33 – SESSION 1.5

Protective role of salicylic acid in ameliorating drought stress in mustard (*Brassica juncea* L.) seedlings by up-regulating the antioxidant defense and glyoxalase system

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The protective role of salicylic acid (SA) was investigated in drought stressed mustard (*Brassica juncea* L.) seedlings. Two sets of 10-d-old seedlings were subjected to two different levels of drought (10% and 20% PEG, 48h), where one set of seedlings was supplemented with 50 µM SA. The relative water content, chlorophyll content decreased at any level of drought. Drought stress caused a sharp increase in proline, MDA and H2O2. Drought stress caused a decline in AsA content and increase in GSH and GSSG content. Compared to control the activities of CAT and MDHAR did not change due to drought stress. The activity of GR slightly increased only at 10% PEG, while APX and GST activity increased at any level of stress. The activities of GPX) and glyoxalase II decreased only at 20% PEG, while DHAR and glyoxalase I activities decreased at any level of stress. On the other hand, supplementation of SA in drought stressed seedlings increased the RWC and Chl content, increased the AsA and GSH, and maintained a higher ration of GSH/GSSG. Salicylic acid supplemented drought stressed seedlings also enhanced the activities of MDHAR, DHAR, GR, GPX, CAT, Gly I, and Gly II, with a concomitant decrease in H2O2, and MDA. These results suggest that the exogenous application of SA assisted the plants to become more tolerant to drought stress-induced oxidative damage by enhancing their antioxidant defense and glyoxalase systems.

POSTER 34 – Session 1.5

Transcriptome analysis of young maize primary roots subjected to drought stress by RNA-Seq

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Water deficit is one of the most severe abiotic stresses affecting plant life. With the climate change being expected to reduce areal water availability breeding for drought-tolerant crop cultivars is recommended. To gain a better understanding of the mechanisms underlying drought tolerance the molecular responses of maize (*Zea mays* L.) primary roots to water stress were investigated. Young maize (B73) seedlings were subjected to low water potentials established with polyethylene glycol (PEG8000) in semi-hydroponic conditions. Utilizing RNA-Seq analyses patterns of differential gene expression of water stressed in comparison to well-watered roots were determined. Results indicate that genes respond in a stress-dependent manner: Reduced water availability or longer stress treatment leads to higher numbers of affected genes. The number of significantly differentially expressed genes varies between a few hundred for short mild stress and thousands for long-term severe stress. Stress-responsive genes are involved in a broad range of cellular and biochemical functions among which protein metabolism and transport processes are over-represented. A distinct set of genes is differentially expressed independently of the nature of stress treatment and represents possible candidates for forward genetic analyses leading to improvement of maize drought-tolerance.

POSTER 35 – Session 1.5

Impact of long-term environmental stress on C-N-P balance of maize source leaves

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Question: Abiotic stress causes disturbances in the cellular homeostasis. Re-adjustment of balance in carbon, nitrogen and phosphorus metabolism therefore plays a central role in stress adaptation. However, it is currently unknown which parts of the primary cell metabolism follow common patterns under different stress conditions and which represent specific responses. Methods: To address these questions, changes in transcriptome and metabolome were analysed in maize source leaves from plants suffering low temperature, low nitrogen (N) and low phosphorus (P) stress. The selection of maize as study object provided data directly from an important crop species and the so far underexplored C4 metabolism. Results: Growth retardation was comparable under all tested stress conditions. The only primary metabolic pathway responding similar to all stresses was nitrate assimilation, which was down-regulated. The largest group of commonly regulated transcripts followed the expression pattern: down under low temperature and low N, but up under low P. Several members of this transcript cluster could be connected to P metabolism and correlated negatively to different phosphate concentration in the leaf tissue. Conclusions: Maize therefore employs very different strategies to manage N and P metabolism under stress. While nitrate assimilation was regulated depending on demand by growth processes, phosphate concentrations changed depending on availability, thus building up reserves under excess conditions.

POSTER 36 – SESSION 1.5

The influence of salt stress on the Zea mays chloroplast transcript pattern

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Soil salinity severely affects growth and yield of salt sensitive crops such as maize. It is also known that salt stress affects the morphology of chloroplasts and limits the photosynthetic efficiency. Previous studies have shown that in the initial phase of moderate salt stress chloroplasts quickly accumulate large amounts of sodium ions. The accumulation of sodium may not only affect proteins but also the chloroplast gene expression. Using a microarray approach we investigated the transcriptional profiles of maize chloroplasts from two different genotypes, one salt sensitive and one salt resistant hybrid, under conditions of different salt stress levels in comparison to those from untreated control plants. We could show that salt stress has a specific influence on the transcript pattern of maize chloroplasts and that salt sensitivity is already detectable at chloroplast transcript level.

POSTER 37 – SESSION 1.5

Global expression analyses between abiotic and biotic stress response in barley.

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Plant development is often affected by one or more external factors like biotic or abiotic stresses. Recent investigations indicate that there is cross-talk between stress responsive pathways, e.g. abiotic and biotic stress responses. Plants are able to respond to stress via regulatory networks triggering changes at transcriptional, cellular, and physiological levels. Global changes in gene expression under different stress conditions were compared using the Agilent 60k-Barley Gene-Array. Abiotic stress was induced using a drought stress system in which plants slowly dry-out, reflecting the natural conditions in the field. Biotic stress was applied by infection with the hemibiotrophic fungus *Bipolaris sorokiniana*. Beside many genes which are specifically up- and downregulated under abiotic or biotic stress, we also found genes affected by both situations, clustering in two groups of co- and counter-regulated genes. We especially focus on regulatory factors in these two groups which might be important switch-points in cross-talk between biotic and abiotic stress. Regulation of these regulatory genes during different abiotic and biotic stress conditions and in response to phytohormones is analysed via qRT-PCR. Furthermore, mutants of two putative cross-talk factors, a NAC transcription factor and a putative HIPP-regulator, were generated. This will allow studying their function in cross-talk between abiotic and biotic stress response pathways.

POSTER 38 – Session 1.5

Cross-talk of polyamine and calcium signalling in stress tolerance of barley (*Hordeum vulgare*)

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Common polyamines, like putrescine, spermidine and spermine, play a central role in abiotic stress tolerance of plants. Spermine appears as a central molecule in drought stress tolerance of the model crop barley (*Hordeum vulgare*). Spermine- and spermidine-synthesizing enzymes were identified in barley. A protocol for polyamine measurement was established using derivatisation and GC-MS separation. Enzyme kinetic characterization is on-going. Drought stress was exerted on barley plants in climate chambers to monitor the gene expression of the enzymes under prolonged drought. First qRT-PCR results show an upregulation of spermine synthase transcripts when water content falls below 40%. For validation further qRT-PCR analyses after drought stress are performed currently. The structural isomer of spermine, thermospermine, regulates stem elongation in *Arabidopsis thaliana*. Barley as a monocot plant has not been investigated for a thermospermine synthase and possible roles of thermospermine in drought stress and stem elongation. Cloning of candidate genes for thermospermine synthase is on-going. In drought stress, stomata are closed by decrease in guard cells turgor mediated by calcium signalling. Thus, we hypothesised that polyamines act *via* calcium and visualised calcium concentrations by Aequorin-based luminescence measurements in epidermal strips of barley leaves.

POSTER 39 – Session 1.5

Cuticular transpiration control of *Rhazya stricta* Decne. in the hot and dry climate of Saudi Arabia

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In arid and semi-arid regions plants require physiological mechanisms in order to survive drought stress conditions. During dry periods plants reduce transpiration by stomatal closure. Under such conditions plant life depends on the efficacy of the cuticular transpiration barrier. Reduction of uncontrolled water loss to the atmosphere enables the plant to maintain an adequate water status. *Rhazya stricta* Decne. (Apocynaceae) is a widely distributed shrub of the desert vegetation in Saudi Arabia, except deep sands. Drought adaptations on the cuticular level were investigated for leaves of *R. stricta*. Cuticular wax composition was analysed by gas chromatography and mass spectrometry. The wax is mainly composed of pentacyclic triterpenoids. Only a minor fraction consists of aliphatic long-chain alkanes. Leaf drying curves were measured to assess minimum (cuticular) water permeability at maximally closed stomata in the dark and under drought stress. The effect of temperature between 25 and 55 °C on cuticular water permeability was analysed by an Arrhenius plot. The absence of a phase transition indicates that the cuticular waxes of *R. stricta* exhibit a special adaptation maintaining the structural integrity of the cuticle to prevent excessive water loss at high temperatures. The high melting points of the triterpenoid constituents of the wax may contribute to this functional adaptation.

POSTER 40 – SESSION 1.5

Differential gene expression of candidate genes for drought stress response in silver fir seedlings

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European silver fir (*Abies alba* Mill.) is an important forest tree species which covers a large geographic range of central and southern Europe. Rising temperatures and decreasing precipitation, as projected for temperate latitudes, in combination with the species' long generation time and its susceptibility to water deficit, might pose a challenge for *A. alba* in the future. Since knowledge is still sparse for conifer species, understanding drought stress response in *A. alba* is of critical importance for future conservation strategies and silvicultural decisions. In an experimental approach, using non-invasive terahertz spectroscopy, the water content of cotyledons from irrigated and water-deprived seedlings was monitored and cotyledons of each treatment group were sampled. A subsequent transcriptome profiling identified candidate genes which potentially play a relevant role in the drought stress response of *A. alba*. A set of those genes was used in a RT-qPCR experiment to validate differential gene expression among stressed seedlings and a control group. First results will be presented.

POSTER 41 – SESSION 1.5

Testing the effect of temperature on carbon allocation of freshwater phytoplankton algae: an FTIR spectroscopy approach

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Temperature together with nutrients and light is one of the main factors that affect phytoplankton growth. Most of the studies conducted on temperature have been performed on huge intervals over a small temperature range with growth rate and photosynthesis as the main target parameter. Temperature can affect membrane fluidity, photosynthesis and enzyme activity. Thus, changes in carbon allocation, i.e qualitative and/or quantitative changes in the main organic cellular pools (protein, carbohydrate and lipid), might be expected as a response to different growth temperatures. Fourier transform Infrared (FTIR) spectroscopy coupled with chemometric analysis have found application in numerous physiological studies conducted on algae, because it allows to rapidly acquire a snapshot of cell organic composition. In order to study the effects of temperature on C-allocation of phytoplankton algae we cultivated four freshwater algal species (Cyanobacteria and Chlorophyta) over a temperature range between 7 and 35°C in 4°C increments and characterized the organic composition by FTIR spectroscopy and chemometrics. Physiological implications, in particular C-allocation will be discussed in terms of energetic budget at the different temperatures. Furthermore, the consequences of different C-allocation patterns will be considered with respect to phytoplankton dynamics.

POSTER 42 – Session 1.5

Vitis sylvestris harbours genetic variation in stilbene metabolism

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Vitis vinifera L. ssp. *sylvestris* (Gmelin) Hegi, the European Wild Grape and ancestor of cultivated grapevine varieties (*V. vinifera* L. ssp. *vinifera*) is the only wild grapevine species existing in Europe. This important Crop Wild Relative (CWR) species is almost extinct and persists only in residual habitats. Recently, CWR have attracted the attention as valuable genetic resources for breeding. Some of the *sylvestris* genotypes harbour valuable resistence factors against several diseases of grapevine, such as Powdery Mildew (*Erysiphe necator*), Downy Mildew (*Plasmopara viticola*), and Black Rot (*Guignardia bidwellii*). However, since they had not been previously exposed to these diseases before, the resistance must be different from conventional effector-triggered immunity. For this reason, we want to know what is the potential mechnism present in CWR species as a genetic resource for breeding in relation to several grapevine diseases. Stilbenes are a small family of plant secondary metabolites, which have implications for plant disease resistance and human health. They are generally involved in the response to biotic and abiotic stresses. In this study, we show that some of the resistant *sylvestris* genotypes show elevated levels of stilbenes in response to UV-C, correlated with elevated induction of metabolic genes in the stilbene synthesis pathway.

POSTER 43 – SESSION 1.5

ARABIDOPSIS PLAT DOMAIN PROTEIN1 IS CRITICALLY INVOLVED IN ABA SIGNALLING AND PLANT STRESS RESPONSES

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Family members of the PLAT-plant-stress subgroup are induced by different stresses, but little information is available on their function. We identified and characterised the *Arabidopsis* PLAT domain protein 1 (PLAT1), which is a member of this subgroup and harbours a single PLAT domain, as novel positive regulator within the ABA signalling pathway. ABA and abiotic stress treatments induced *PLAT1* expression, phenotypic changes related to PLAT1 function are similar to altered ABA signalling and the *PLAT1* promoter is a direct target of the ABF transcription factors. Concomitant with its function in ABA signalling, PLAT1 loss-of-function reduced abiotic stress tolerance, whereas gain-of-function conferred increased abiotic stress tolerance, but reduced tolerance towards biotic stress. In addition, plant growth was promoted by PLAT1 gain-of-function in both *Arabidopsis* and tobacco. PLAT1 is localised in the endoplasmic reticulum (ER) and functions in ER stress responses, probably through brassinosteroid signalling. Thus, we have identified a critical function for PLAT1 in the ABA signalling pathway, promoting both plant abiotic stress responses and plant growth.

POSTER 44 – Session 1.5

The Oxidative Stress-Responsive factor ZAT12 Modulates Iron Deficiency Responses by Interacting with the Central Regulator FIT

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Under limiting iron supply conditions plants induce a set of genes whose products enhance the iron uptake efficiency of the roots. A central regulator of this response is the basic helix-loop-helix transcription factor FIT whose activity is tightly regulated at transcriptional and posttranscriptional level. Using a yeast two-hybrid approach, we identified the zinc-finger transcription factor ZAT12 as a FIT interacting partner. We investigated the regulation of *ZAT12* at both gene and protein expression level under different iron supply regimes. The lack of functional *ZAT12* leads to upregulation of *FIT* gene expression. Furthermore, under sufficient iron supply *ZAT12* loss-of-function plants accumulate higher amounts of iron in their shoots compared to wild type plants. Taken together our results suggest that ZAT12 acts as a negative regulator of FIT and iron deficiency responses. Since the *ZAT12* gene responds to oxidative stress, it might represent a link between iron deficiency response and reactive oxygen species signaling.

POSTER 45 – Session 1.5

Genetics of Low Temperature Response of Photosynthesis and Growth in Brassicaceae

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Temperature is an important factor determining plant growth, performance and geographic distribution. Hence it has a high agronomic impact. One of the first physiological parameters that is affected by low temperature is photosynthetic efficiency. Natural variation for photosynthetic efficiency in the cold has been reported, suggesting different adaptation strategies. However, very little is known about the mechanisms involved in regulation of photosynthesis in cold and the underlying genetics. We use photosystem II efficiency, determined by chlorophyll fluorescence imaging, as an indicator for low temperature stress. An automated phenotyping system allows precise and high-throughput measurement of plant growth and chlorophyll fluorescence. In this way we obtain a unique dataset that provides information about the physiological status of the plants over the entire stress period. We detected quantitative trait loci (QTL) with a *Brassica rapa* recombinant inbred line population. Among them a major QTL (high LOD-score and high explained variance) which was unexpected for a supposedly conserved trait as photosynthesis. An association mapping with a densely genotyped collection of *Arabidopsis thaliana* natural accessions revealed one significantly associated single nucleotide polymorphism pinpointing to a gene that might be involved in cold sensing.

POSTER 46 – Session 1.5

UV-B damage to Ulva intestinalis in the field

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UV-B radiation (280-315 nm) damages DNA by inducing the formation of cyclobutane pyrimidine dimers (CPDs) and by inhibiting the oxygen evolving complex at photosystem II (PS II). Plants protect themselves against the detrimental effects of UV radiation by the accumulation of screening pigments. However, as many other green algae, the macroalga *Ulva intestinalis* (Chlorophyta) does not possess UV screening compounds. On the other hand, this species is very successfully colonizing the eulittoral, where it is periodically exposed to strong solar radiation including UV-B. Especially the fact that thalli can be observed floating on the water surface in full sunshine seems to speak for excellent resistance against solar radiation. In laboratory experiments, *U. intestinalis* showed a dose-dependent inhibition of PS II quantum yield (Fv/Fm) and induction of CPDs after exposure to realistic irradiances of UV-B. Therefore, it is interesting to assess damage to PS II and CPD accumulation under natural conditions. Preliminary field data showed significant photoinhibition during a sunny day, but concomitant increases in CPDs were lacking. The results of an experiment exposing *U. intestinalis* in mesocosms under UV screening Perspex shields will be reported. The relationship between damage to PS II and CPD accumulation will be analyzed.

POSTER 47 – SESSION 1.5

Calcium signalling in guard cells - K+-efflux channels as rate-limiting elements in jasmonate-triggered stomatal closure

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Stomatal aperture and thus plant water use efficiency is controlled by guard cells integrating multiple environmental and endogenous signals. The phytohormone ABA represents the paradigm signaling pathway for stomatal closure. Similar to ABA, Jasmonic acid (JA) mediates fast stomatal closure. The molecular chain of events underlying this phenomenon however is largely scant. We here show that the guard cell K+ efflux channel GORK represents an essential target during fast JA-mediated stomatal closure. JA action requires activation of GORK by the kinase CIPK5 together with its Ca2+-sensitive activators CBL1 and CBL9. Additionally, in the ground state, GORK activity appears suppressed by the phosphatase ABI2 providing a link between ABA and JA signalling in guard cells. Consistent with a role of K+-release during fast stomatal closure this antagonistic phosphatase/kinase relay complex bridges phytohormone sensing and volume regulation in *Arabidopsis* guard cells.

POSTER 48 – SESSION 1.5

Some physiological and biochemical effects of exogenous proline on durum wheat (*Triticum durum*) subjected to a salt constraint

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Plants are often confronted with abiotic stresses such as salinity which is one of the main environmental problems affecting growth and productivity particularly in arid and semi-arid areas. At the physiological level, salinity imposes ion toxicity and an osmotic stress that limits water uptake in plant. To prevent water loss from the cell and to sustain cellular functions under saline constraint, plants accumulate a number of compatible solutes. Among these, proline has been reported to be increased naturally in stressful conditions and constitute the major metabolites found in most crop species in response to salt stress including durum wheat. Our study shows the effects of exogenous proline (20 mM) on the physiological and biochemical behavior of durum wheat seedlings (Triticum durum) subjected to a salt constraint induced by 10 g.L-1 of sodium chloride. The results obtained show that the saline stress affects the majority of the studied parameters. However, the exogenous application of proline seems to attenuate these negative effects of the saline stress by the improvement of the contents of total chlorophylls, water soluble proteins and glycine betaïne. These results suggest the capacity of the exogenous proline to improve the tolerance of the plants subjected to salt constraint.

Key words: exogenous proline, salt stress, wheat,osmotic adjustment, catalase

POSTER 49 – SESSION 1.5

Investigations of the auxin response to salt stress by the moss *Physcomitrella patens*

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The moss *Physcomitrella patens* is an important model organism to investigate different types of abiotic stresses and their influence on cellular processes and molecular pathways. The gametophytic tissue of this plant contains one native auxin indole-3-acetic acid (IAA). In order to investigate the effects caused by the salt as abiotic factor, two mutant lines in *GH3* genes were compared with *Physcomitrella* WT plants on medium supplemented with different concentrations of NaCl. The GH3 proteins are responsible for the inactivation of IAA by conjugation to various amino acids. The salt stress response was assessed at first by measuring the growth area for each sample during a 40-50 day growth period. In a second experimental approach the auxin response and distribution was indirectly characterized in *P. patens* using an auxin-inducible reporter gene system (GH3::GUS line). The results indicate an involvement of auxin homeostasis in the response to high salt concentrations.

POSTER 50 − SESSION 2.1

Flg22 sensors in cortex cells trigger Ca2+-mediated sieve-tube occlusion

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Plants, like animals, sense microbial invaders by using pattern-recognition receptors such as FLS2 to recognize microbe-associated molecular patterns (MAMPs). Perception of MAMPs, among others triggers signals that reach the phloem to promote sieve element (SE) occlusion. Rapid SE occlusion forms a physical barrier to restrict pathogens and to accumulate signal molecules-reversibility ensures a systemic spread of signals. Flagellin (flg22)-triggered SE occlusion was observed in *A. thaliana* plants using confocal laser scanning microscopy and carboxyfluorescein. Absence of SE occlusion in flg22-insensitive mutants indicated SE occlusion as part of the MAMP-triggered immunity. SE occlusion observed in intact *V. faba* plants by dispersion of forisomes after flg22 treatment reveals that Ca2+ is involved in SE occlusion. Absence of forisome dispersion in SE protoplasts and immunostaining of the FLS2 receptor in *V. faba* indicate that the receptor is not located in the SEs. The apparent exclusive presence of FLS2 receptors in cortex cells questioned the mode of signal transfer to the SEs. Electrophysiological measurement of cortex, phloem parenchyma cells and SEs suggests that electropotential waves are transferred from cortex to SEs conferring sieve element occlusion. Thus, we assume that MAMP-triggered SE occlusion is part of the plant´s defense strategy against bacterial pathogens.

POSTER 51 − SESSION 2.1

Direct and individual analysis of stress-related phytohormone dispersion in the vascular system of *Cucurbita maxima* after PAMP treatment.

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Various phytohormones have been identified as important components of signalling cascades in plant development and response to various (a)biotic challenges. Although many studies have emphasized the role of jasmonates, salicylic acid (SA) derivatives, and abscisic acid (ABA) in defences, the nature of the long-distance signal(s) is still controversial, at least in part because little is known about the distinct role of the translocation pathway - the vascular system. Phloem and xylem exudates of *Cucurbita maxima* were separately collected and the stress-related phytohormones SA, ABA, and the three jasmonates, jasmonic acid (JA), *cis*-12-oxo-phytodienoic acid, (+)-7-*iso*-jasmonoyl-L-isoleucine (JA-Ile) were analysed via liquid chromatography - mass spectrometry. We show direct evidence for all phytohormones in both phloem and xylem exudates. JA and JA-Ile concentrations are higher in xylem, whereas ABA and SA concentrations are higher in phloem exudates. During bacteria-derived flg22-triggered remote root-to-shoot signalling, phytohormone concentration changed rapidly both in phloem and xylem. The unequal distribution of phytohormones suggests that phloem and xylem have distinct roles in defence responses. Our data shed light on systemic phytohormone signalling and help explain how plants cope with environmental challenges by lateral exchange between phloem and xylem.

POSTER 52 − SESSION 2.1

Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid

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Lipid peroxidation (LPO) is induced by a variety of abiotic and biotic stresses, however, little is known about the oxidation mechanisms and major lipid targets. A systematic lipidomics analysis of LPO in the interaction of *Arabidopsis thaliana* with *Pseudomonas syringae* revealed that LPO is predominantly confined to plastid lipids and precedes programmed cell death. Singlet oxygen was identified as the major cause of lipid oxidation under basal conditions while a 13-lipoxygenase (LOX2) and free radical-catalyzed lipid oxidation substantially contribute to the increase upon pathogen infection. Analysis of *lox2* mutants revealed that LOX2 is essential for enzymatic membrane peroxidation but not for the production of free jasmonates in response to pathogens. Pathogen infection also induced an accumulation of fragmented lipids. We provide strong *in vivo* evidence for a free radical-catalyzed galactolipid fragmentation mechanism responsible for the formation of the essential biotin precursor pimelic acid as well as of azelaic acid, which was previously postulated to prime the immune response of *A. thaliana.* Our results suggest that azelaic acid appears to be a marker of LPO rather than a general immune signal. However, the proposed fragmentation mechanism rationalizes the pathogen-induced radical amplification and formation of electrophile signals such as phytoprostanes, malondialdehyde and hexenal in plastids.

POSTER 53 – Session 2.1

Rhizobacterial volatiles effecting *Arabidopsis thaliana*

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Soil-borne, root-associated microorganisms are an important source of diverse volatile metabolites. These volatiles possess the potential to affect plant´s vitality without direct contact (1).

The response of *Arabidopsis thaliana* to volatile blends of *Serratia plymuthica* and *Stenotrophomonas maltophilia* was analysed in detail at the physiological, transcriptional and metabolic level. Significant inhibition of plant growth was observed already after 2 days in contact-free co-culture accompanied by systemic H2O2 production and chlorosis. Microarray analysis at 6, 12 and 24 hours of co-cultivation showed a regulation of ca. 1000 genes. 162 genes were commonly activated by both bacterial volatile blends. Among these up-regulated genes, the transcription factor *wrky* 18 turned out to play a key role in the plant defence mechanism in co-culture. Individual compounds of the volatile blends, e.g. such as 2-phenylethanol and DMTS, were applied to *A. thaliana* in a dose dependent manner to determine IC50 concentrations.'

1 Vespermann, A et al. 2007. Appl and Environm Microbiol 73:5639-5641

POSTER 54 – Session 2.1

AtCPK5 acts as multi-functional defense regulator upon pathogen attack

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Calcium influxes are known to be one of the primary and highly conserved events in several plant stress responses. The stimulus-dependent variations of calcium signatures require decoding by signaling molecules initiating respective downstream signaling events. Calcium-Dependent Protein Kinases carry 4 EF-hands in their Calmodulin-like domain and a protein kinase domain within one molecule. CDPKs were shown to play a major role in abiotic, as well as biotic stress signaling and are thus predestinated to function as calcium decoders in plant signaling responses. Here we investigate a distinct isoform from the *Arabidopsis thaliana CDPK* gene family which links primary signaling events with the activation and regulation of late downstream responses. The multifunctional protein could be shown to be a positive regulator of early PAMP-triggered defense signaling (1). Additionally CPK5 seems to be involved in phytohormone crosstalk required for late response regulation and disease resistance. Unexpectedly we could comprise a third independent function linking CPK5 signaling upon PAMP perception to the establishment of systemic resistance. It is thus conceivable to postulate a key regulatory role of this isoform in tightly regulating defense responses upon pathogen attack.

(1) Dubiella et al., 2013 doi: 10.1073/pnas.1221294110

POSTER 55 – SESSION 2.1

Biosurfactants of *Pseudomonas syringae* enhance nutrient leaching through isolated cuticles of ivy (*Hedera helix*) and poplar (*Populus canescens*)

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The plant cuticle represents a harsh environment for epiphyllic bacteria. Cutin associated waxes are hydrophobic. Thus leaf surfaces are non-wettable and colonization by epiphyllic bacteria is hampered. Furthermore, nutrient availability is low because the plant cuticle represents an efficient barrier for water and nutrient loss. Many epiphyllic bacteria produce biosurfactants, increasing leaf surface wettability and mobility of bacteria on the leaf surface. The potential effect of biosurfactant producing (WT) and non-producing *Pseudomonas syringae* bacteria (MT) on water and solute diffusion across ivy (*Hedera helix*) and poplar (*Populus canescens*) cuticles was investigated. 3H-water permeability of poplar cuticles was significantly higher compared to ivy cuticles. Inoculation of cuticles with *P. syringae* WT and MT did not affect water permeability. However, 14CO2-evolution of metabolized 14C-labelled arginine and fructose diffusing through the cuticle was higher when cuticle surfaces where inoculated with biosurfactant producing *P. syringae* WT compared to MT. This allows to conclude that biosurfactants can enhance leaching of nutrients through the cuticle and thus increase nutrient availability for epiphyllic bacteria.

POSTER 56 – Session 2.1

Phosphoinositide-signals in plant-pathogen interactions

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Upon infection by pathogenic microorganisms, plants induce defense responses including the exocytosis of antimicrobial compounds and secreted callose. Therefore, exocytosis is an important aspect of plant-pathogen-interactions. The defensive measures of an infected host plant are countered by microorganisms via the production of effector proteins that suppress host defenses. The pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) uses a type III secretion system to translocate a multitude of effector proteins (*Xanthomonas* outer proteins, Xops) into pepper, tomato and tobacco plants to modulate plant basal defense. The focus of this study is the influence of the effector protein XopJ. XopJ is a member of the YopJ family of SUMO peptidases and acetyltransferases and has been demonstrated to inhibit membrane trafficking in plant cells. Several lines of evidence suggest that XopJ targets the host phosphoinositide (PI)-system. PIs are membrane phospholipids with broad regulatory effects on numerous cellular processes with relevance for pathogen defense. Here we show that XopJ has an effect on PI-mediated cytoskeletal dynamics and secretion.

POSTER 57 – SESSION 2.1

An Arabidopsis chitinase hydrolyses peptidoglycan to enhance plant innate immunity to bacterial infection

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Peptidoglycans (PGNs), essential bacterial cell surface patterns are recognized in Arabidopsis by plant cell surface localized receptors to activate the plant innate immune response including medium alkalinization, transcription of defense-related genes and accumulation of callose deposits (1,2). However, due its complex structure PGNs are likely to be processed into smaller fragments to cross plant apoplastic matrix prior to recognition via corresponding plasma membrane receptors. Here, we found that the Arabidopsis chitinase CHIA habours PGN-degarding activity. This class III chitinase CHIA is located in the plant apoplast and is transcriptionally up-regulated upon PGN treatment. CHIA has chitinolytic as well as peptidoglycanolytic activity, and can cleave PGN from both Gram-negative and Gram-positive bacteria. CHIA-released soluble PGN fragments can activate the plant immune response such as pH increase and FRK1 upregulation. Most importantly, genetic inactivation of CHIA renders Arabidopsis plants more susceptible to bacterial infection. Surprisingly, CHIA over-digested PGN fragments impair their ability to trigger plant immune responses which is in accordance with the fact CHIA-overexpressing Arabidopsis plants are more susceptible to bacterial infection

POSTER 58 – Session 2.1

Plant Receptor Kinases as Targets for Bacterial Effectors

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Pathogenic bacteria have evolved multiple ways to facilitate the infection of their respective host plants. Co-evolution of plant defense mechanisms has led to an evolutionary arms race where secreted bacterial proteins, termed effectors, target various plant proteins to suppress plant innate immunity. In turn, these effectors can be recognized by specialized plant resistance proteins. The bacterium *Pseudomonas syringae* pv. tomato, a well-studied natural pathogen of tomato, can use a battery of effector proteins to aid in its virulence. One of these effectors, termed AvrPto, has in previous studies been described as an interactor of various receptor-like kinases in the model plant *Arabidopsis thaliana*. Among these are FLS2, the plant flagellin receptor, as well as BAK1, a protein with multiple roles including innate immunity and brassinosteroid signaling. There is disagreement upon which plant protein is the naturally favored target of AvrPto. To aid in understanding the biochemistry and evolutionary background of this bacterial effector, interaction studies including AvrPto as well as various heterologously expressed and purified kinase domains of *A. thaliana* receptor-like kinases will be performed. Determination of quantitative binding affinities should give an idea about which part of the complex innate immunity signaling cascade is of special importance for bacterial virulence.

POSTER 59 – SESSION 2.1

Mechanistic studies of the BAK1-interacting RLK BIR2

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Plants have to deal with different environmental stress situations such as heat, osmotic stress or biotic pathogens. Therefore, it is necessary to have well-adapted protection mechanisms starting with the recognition of invaders by plasma membrane-located receptors. BAK1 (BRI1-associated kinase 1) is a leucine-rich repeat receptor-like kinase (LRR-RLK) and central regulator of ligand-binding receptors involved in diverse processes such as brassinosteroid signaling, plant innate immunity and cell death control. BIR2, a recently identified LRR-RLK is a constitutive interactor of BAK1 that differentially influences BAK1-regulated signaling pathways by negatively regulating complex formation of BAK1 with ligand binding receptors. My studies are focused on further interactor partners of BIR2 including other leucine-rich repeat receptor kinases and the elucidation of the phosphorylation dependent mechanism of BIR2 function. BIR2 is an atypical inactive kinase but is unidirectionally phosphorylated by BAK1. First results on the relevance of these phosphorylation events on its function in vivo will be presented.

POSTER 60 – SESSION 2.1

Molecular and functional analysis of the BAK1 interacting protein BIR2

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BAK1, the BRI1-associated kinase1, is a leucine-rich repeat receptor-like kinase (LRR-RLK) that interacts with several ligand-binding RLKs to positively regulate their functions. Identification of *in vivo* BAK1 complex partners by LC/ESI-MS/MS uncovered two additional BAK1-interacting RLKs, BIR2 and BIR3. Phosphorylation studies revealed that BIR2 is unidirectionally phosphorylated by BAK1 and that the interaction between BAK1 and BIR2 is kinase-activity dependent. Functional analyses of *bir2* mutants show differential impact on BAK1-regulated processes, such as hyper-responsiveness to pathogen associated molecular patterns (PAMP), enhanced cell death and resistance to bacterial pathogens, but no effect on brassinosteroid-induced responses. BIR2 interacts constitutively with BAK1 thereby preventing interaction with the ligand-binding LRR-RLK FLS2. Treatment with PAMPs leads to the release of BIR2 from the BAK1 complex and recruitment of BAK1 into the FLS2 complex. Our results provide evidence for BIR2 acting as a negative regulator of PAMP-triggered immunity by limiting FLS2-BAK1-complex formation in the absence of the ligand.

POSTER 61 – SESSION 2.1

Unraveling the molecular mechanism behind bZIP10-LSD1 interaction in *Arabidopsis thaliana*

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Transcription factors containing a conserved stretch of basic amino acids followed by an alpha-helical structured leucine zipper domain constitute the bZIP protein family. Various physiological and developmental processes in plants are regulated by bZIP transcription factors. These include leaf and seed formation, energy homeostasis, photomorphogenesis, and biotic and abiotic stress responses. bZIP10, a member of the C-group bZIP transcription factors, was shown to interact with LSD1 (Lesion Simulating Disease 1), which is a negative regulator of plant cell death. Through this interaction, bZIP10 is involved in the superoxide-induced cell death which is essential for plant hypersensitive response (HR) upon pathogen attack (Kaminaka et al. (2006) *Embo J* 25: 4400-4411). However the mechanism of bZIP10 regulation and the nature of the signal governing bZIP10-LSD1 interaction are largely unknown. We show here that bZIP10-LSD1 interaction could be dependent on the redox state of the cell. As demonstrated by mutational analysis, the cysteine at position 409 in bZIP10 seems to be the key player. The modification of C409 residue leads to its reduced interaction with LSD1. Furthermore, the antagonistic nature of the functions of bZIP10 and LSD1 is also shown in the reporter gene studies. Therefore, it appears LSD1 regulates the activity of bZIP10 in a redox dependent signaling mechanism.

POSTER 62 − SESSION 2.2

Phosphorylation is a Key Mechanism in stress-induced Callose Biosynthesis

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The deposition of the (1,3)-ß-glucan callose in papillae, cell wall thickenings at sites of attempted pathogen penetration, is an early plant defense response to prevent or slow pathogen ingress.

In *Arabidopsis thaliana*, the callose synthase GSL5 is responsible for callose biosynthesis after stress. Despite the importance of GSL5 in early defense response, only little is known about its regulation. Based on results in yeast that indicated the involvement of phosphorylation in regulating callose biosynthesis, we mutated a serine (S), which was identified to be phosphorylated after stress, to an alanine (A) to prevent phosphorylation at this residue of GSL5. In contrast to the overexpression of the native GSL5, the newly generated *A. thaliana* lines with an overexpression of the S->A mutated GSL5 did not show resistance to the powdery mildew *Golovinomyces cichoracearum*. Moreover, callose deposition was even decreased in these lines compared to wild-type. We did not detected the typical callose deposit at the fungal penetration site 6 h post-inoculation in the GSL5 S->A overexpression lines. Also 24 h post-inoculation, only slight callose deposition was observed at penetration sites in contrast to the massive callose accumulation in the wild-type. The results clearly indicate that phosphorylation at the examined residue is involved in the regulation of GSL5 activity after biotic stress.

POSTER 63 − SESSION 2.2

Functional analysis of antibacterial peptides from *Ginkgo biloba*

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Plants are exposed to a variety of potential threatening pathogens and pests. As a result, they have evolved different mechanisms for self-defence, such as the production of secondary metabolites and defence-related proteins. *Ginkgo* as a 'living fossil' represents one of the oldest gymnosperm species and harbours a broad spectrum of resistance or tolerance to many pathogens. Several peptides have been isolated and cloned from *Ginkgo* leaves and seeds that mediate anti-bacterial, anti-fungal, and antiviral effects. A novel antifungal proteins ginkbilobin-2 have been purified and cloned from *Ginkgo* seeds. It consists of 134-aa with a potential signal peptide (26 residues) and inhibits the growth of plant and human pathogenic fungi such as *Fusarium oxysporum* and *Candida albicans* (Sawano et al.,2007). In the current project, we fuse a GFP tag to the peptide and observe the localization in BY-2 cells. Also we will synthesize the peptides fusing it to cell-permeating carriers which can be used to carry cargo across the cellular membrane into living cells and test their cellular responses. Then Apply to different target cells in the context of pathogen responses. Our primary objective is to find new bioactive compounds that can then be optimized in subsequent research.

POSTER 64 – Session 2.2

Biochemical characterization of proteins from *Plasmodiophora brassicae*, the causal agent of clubroot disease

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The obligate biotrophic plant pathogen *Plasmodiophora brassicae* causes the clubroot disease within the family of *Brassicaceae* that include many commercially important agricultural crops like cabbage, radish and oilseed rape. Plant hormones are important signals during the infection and colonization of plants by *P. brassicae*. Auxins and cytokinins act in the regulation of cell elongation and cell division to create the space in the host tissue that is needed for the propagation of the pathogen. Moreover cytokinins are involved in the generation of a metabolic sink to ensure the nutrition of the pathogen. Salicylic acid ant its methyl ester play a very different role and act in plant defense. The finely tuned regulation of these hormone levels seems to be the key for the efficient colonization of the host plant. Until now only a few proteins from *P. brassicae* are functionally characterized. Based on yet unpublished sequence date we have chosen some *P. brassicae* genes for further characterization. The corresponding proteins show homologies to auxin conjugate synthetases, tRNA isopentenyltransferases and methyltransferases. First results of the biochemical characterization of some of these proteins are presented.

POSTER 65 − SESSION 2.2

Towards understanding a new apple tree decline in South Tyrol

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In 2007 a new form of apple tree decline was first observed in the southern lowlands of South Tyrol. Groups of trees exhibit chlorosis, small fruits and within 24 months dieback occurs. Soil analyses showed no aberration regarding pH, nutrients, salinity or heavy metals. Cress biotest revealed a possible involvement of oomycetes. Temperature and soil humidity profiles of affected sites match beneficial conditions for oomycete infections. Therefore, isolation and molecular characterization of oomycetes present in roots of diseased plants was initiated. We present first results of biotests and oomycete identification through sequence analyses.

POSTER 66 – Session 2.2

Recognition of dodder "parasite associated molecular patterns" (parAMPs) by tomato

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Plants of the genus *Cuscuta* (dodder) live as holoparasites with a wide spectrum of potential host-plants. After winding around the host-plant's stem, *Cuscuta* penetrates the tissue with haustoria to connect to the vascular tissues. The parasite then sucks out nutrients, water and carbohydrates from its victim. Successful control of *Cuscuta* is limited to agricultural strategies prior to attachment. Once the interaction between host plants and parasite is established, yield loss will occur, and control methods also damage the crop plants1. For controlling *Cuscuta* at this stage a better understanding of the interaction of *Cuscuta* with its hosts is crucial, especially of those resistant to *Cuscuta*. Surprisingly cultivated tomato (*Solanum lycopersicum*) has a means to fight *Cuscuta*, which its wild relative *Solanum pennellii* has not. Through Introgression lines2 of *S. lycopersicum* with pieces of *S.pennellii* two loci in the tomato genome were identified as important for resistance and hence the receptor-like protein CURE1 was identified to perceive a small glycopeptide parAMP purified from *Cuscuta reflexa*.

1 Lanini and Kogan 2005, 2Eshed et al 1992

POSTER 67 – SESSION 2.2

Generation of novel DAMPs as mobile signal of danger by cytotoxic NEP1-like proteins

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The activation of innate defense mechanisms of plants against microbial infection is mainly based on two branches: the PRR-mediated recognition of PAMPs, (PAMP-triggered immunity) and the recognition of microbial effectors by receptors encoded by R-genes (effector-triggered immunity). Besides these two mechanisms of pathogen perception, plants can also sense endogenous patterns, representing damage-associated molecular patterns (DAMPs), which induce innate immunity. Such endogenous elicitors so far comprise cell wall fragments, cutin monomers and peptides like systemin and AtPEP1. Well-known triggers of plant immune responses are necrosis and ethylene-inducing peptide 1-like proteins (NLPs). NLPs are virulence-promoting toxins found in phytopathogenic bacteria, oomycetes and fungi. By disrupting the plasma membrane of dicotyledonous plants, NLPs are inducing cell death and thus contribute to the virulence of necrotrophic and hemibiotrophic plant pathogens. Studies with active and inactive mutant versions of the NLP from *Pectobacterium carotovorum* showed, that not the NLP molecule itself is recognized, but its membrane disrupting activity. Thus, it is very likely that the activity of NLPs induces the production of breakdown products or the release of intracellular molecules that are sensed as DAMPs. The identification of those plant-derived DAMPs and their corresponding receptors will help to elucidate this novel form of plant innate immunity.

POSTER 68 – Session 2.2

Investigation of the effect of plant strengthener (Frutogard) on phytopathogenic fungi in vitro and on plants

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Plant diseases cause high economic losses. Therefore, the control of plant pathogenic fungi is very important. In order to protect the environment, biological and ecologically acceptable control of plant pathogens has been a considerable topic for research. Regarding the importance of this control method, in this survey we will study the effects of a plant strengthener on the control of fungal plant pathogens both in vitro and on plants under greenhouse conditions. This plant strengthener is a liquid formulation. In preliminary experiments different concentrations of Frutogard are tested with a range of fungi. Comparing the growth behavior of the fungi on the plant strengthener with control medium will help to answer the following questions: A) Is the fungal growth completely stopped? B) Does the fungus show abnormal growth as result of stress? C) Or is fungal growth not affected at all? D) How does Frutogard induce tolerance or resistance in plant to pathogenic fungi? In the next stage we will test the control of the plant pathogenic fungi on plants by Frutogard.

POSTER 69 – SESSION 2.3

Functional analysis of *Arabidopsis FMO2* and *FMO1* expands the roles of flavin-containing monooxygenases to seedling growth and interaction with herbivores

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Flavin-containing monooxygenases (FMOs) form a family of 29 genes in *Arabidopsis* of which 27 are likely to be functional. Analysis of a number of them revealed that these enzymes are involved in as different functions as auxin biosynthesis, defence against microbial pathogens or glucosinolate biosynthesis. We report here the functional analysis of *FMO2*, encoded by the gene at locus *At1g12200*. Our studies indicate that *FMO2* is expressed in all tissues and promoter-GUS reporter lines indicate a phloem-specific expression. Using T-DNA insertion as well as overexpression lines we could show that deregulation of *FMO2* affects early seedling growth and insect larvae feeding choice. Thus, lines overexpressing FMO2 reached an advanced developmental stage compared to wildtype seedlings while *FMO2* T-DNA insertion seedlings were slightly slower developing. Moreover, those lines were clearly preferred by *Pieris rapae* larvae over wildtype plants. Interestingly, *FMO1*-overexpressing lines were mostly avoided by the larvae. Thus,our studies increase the already many physiological functions to which FMOs contribute in plants.

POSTER 70 – SESSION 2.3

Calcium sensor CML37 - a player in herbivore induced plant defense

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Throughout their life, plants are challenged by various abiotic and biotic factors. Next to stresses from the abiotic environment like drought or chemical stimuli, plants will encounter herbivorous insect attacks. The recognition process in plant-herbivore interactions and the signal transduction pathways connecting it to downstream defense induction are poorly understood. Since cytosolic Ca2+ elevations due to stress signals are rather ubiquitous, a proper decoding of these signals is of great importance. One class of Ca2+ sensors which are involved in this are the Calmodulin-like proteins (CMLs). In Arabidopsis, *AtCML37* is induced both by mechanical wounding as well as elicitors in the oral secretion of generalist herbivore *Spodoptera littoralis*. Knock out of At*CML37* leads to a better performance of larvae suggesting that AtCML37 is a positive defense regulator in Arabidopsis. While there are no changes in secondary metabolites like glucosinolates, the production of jasmonates and expression of JA-responsive genes is significantly reduced. AtCML37 is a sensor relay protein which has no enzymatic activity. After binding of Ca2+ and conformational changes it can interact with other proteins. To understand the role of AtCML37 and its position in the signaling cascade, a co-immunoprecipitation protocol is established.

POSTER 71 – SESSION 2.3

A role of phytohormones in carnivory in pitcher plants

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Carnivory in plants is an adaptation to nutrient-poor environments. Carnivorous plants obtain additional mineral nutrients by trapping and digesting (insect)-prey with specialized organs. The species of the genus *Nepenthes* possess pitchers, metamorphosed leaves that contain a digestive fluid inside. This fluid is secreted by bifunctional glands that, in addition, take up the nutrients. Various digestive proteins have been identified in the pitcher fluid. For many of which their corresponding genes were cloned, heterologously expressed, and further characterized. Besides hydrolytic activities, some of these proteins show antimicrobial properties. Although there is an increasing number of reports on the protein compositions of the fluid in *Nepenthes* species, our knowledge about the regulation of the protein secretion and its composition are still limited. Phytohormones play a key role in defense regulation and development in plants. We are interested in the involvement of phytohormones in carnivory. We found that some phytohormones such as salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and related JA metabolites, such as jasmonoyl-*L*-isoleucine (JA-Ile) and 12-hydroxyjasmonic acid (12-OH-JA), can be detected directly in the pitcher fluid. In parallel, the role of phytohormones in developmental processes of the pitcher will be studied.

POSTER 72 – SESSION 2.3

Cabbage and the cabbage white: Cyanide detoxification in Pierids as one element of their coevolutionary relationship

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The Small Cabbage White butterfly, *Pieris rapae* (Lepidoptera: Pieridae), is a specialist on plants defended by the glucosinolate-myrosinase system which generates toxic isothiocyanate upon tissue disruption. Larvae of *P. rapae* and related Pierids express a gut nitrile specifier protein (NSP) to redirect glucosinolate hydrolysis to form nitriles instead. Nitriles derived from aromatic glucosinolates are further metabolised in the larval body giving rise to the formation of cyanide1. As aromatic glucosinolates were widespread in ancient Brassicales, the ability to detoxify cyanide may have been essential for the Pierid's host plant shift to Brassicales. We have investigated the presence and efficiency of cyanide detoxification pathways in *P. rapae*. Larvae were exposed to cyanide through cyanogenic diet or fumigation. Metabolite analyses and enzyme assays showed that β-cyanoalanine synthase (βCAS) and rhodanese (Rho) function in cyanide metabolism1. Activities were detected in all larval stages and proved to be highest in the larval gut. The analysis of other Pierid and non-Pierid species showed that only those exhibiting NSP activity2 had high levels of both βCAS and Rho. In *P. rapae*, a degenerate-primer based approach to detect mRNA sequences of βCAS has yielded three candidate sequences for further analysis.

1 PloS one (2012) 7(4): e35545

2 PNAS (2007) 51:20427-20431

POSTER 73 – SESSION 2.3

Role of defence phytohormone jasmonic acid in root-knot nematode resistance

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Root-knot nematodes (*Meloidogyne spp.)* are plant-parasitic nematodes which have a broad host range; hence, they are considered to be one of the major threats to cultivated plants. During a compatible interaction in the root, they will choose several plant cells and convert them into multinucleated cells (giant cells). The infection causes galling which eventually will disturb the root vascular system, and, therefore, reduces plant growth and crop production. It has been shown that some plant hormones changes plant susceptibility to root-knot nematodes. However, the mechanisms by which phytohormone signaling and downstream responses can alter plant susceptibility to root-knot nematodes are poorly understood. By using *Arabidopsis thaliana* as a model plant, we are studying the roles of particular phytohormones during the interaction with the model root-knot nematode *Meloidogyne hapla*. In this work, we are primarily interested in the defence hormone jasmonic acid and its role in induced nematode defence. By using jasmonic acid mutants and methyl jasmonate treatment, our initial findings suggest a role of jasmonic acid in inducing nematode defence, but that this JA-induced resistance occurs independently of the JA-receptor COI1 in Arabidopsis. By using different approaches with the resources available for Arabidopsis, we would like to further investigate the mechanism behind this resistance.

POSTER 74 – SESSION 2.3

Analysis of potential effectors in root-knot nematode - plant interaction

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Root-knot nematodes (*Meloidogyne spp.*) are obligate biotrophs that establish an intimate relationship with their host plant by creating a feeding site. The establishment of a feeding site results in the formation of a "gall" which is a swelling of the root due to intensified cytogenesis around the feeding sites. By interacting with the plant host these roundworms can severely reduce agricultural and horticultural production. At the molecular level, the compatible interaction between host and parasite is not completely clear. Nematodes are able to pierce through the plant cell wall and plasma membrane with a straw-like mouth structure called the stylet. Through the stylet, they can secrete molecules into the plant cytoplasm that can to manipulate the plant cells Some of these proteins, called effectors, may down regulate plant defenses during the susceptible interaction. In order to identify and characterize novel effectors, we are using different molecular tools e.g. gene expression analyses, *in situ* hybridization and a heterologous system using transgenic *Pseudomonas syringae.* Here we will present our initial findings of our effector candidate search and discuss candidate(s) involved in the root-knot nematode- *Arabidopsis thaliana* interaction. Knowledge about the function of these proteins might lead to the development of more effective plant protection methods.

POSTER 75 – SESSION 2.3

Alternation of nitrate fertilization can twist plant-mediated herbivore interactions

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Plants face and respond to a variable environment characterized by different nutrient availability in the soil and biotic factors like herbivory, which potentially induce local and systemic modifications in the plant metabolome. Likewise, herbivores depend on the conditions of their hosts as the nutritional status of the plant and feeding by other, even spatially separated herbivores can affect them considerably in a positive or negative way. Abiotic factors like plant nutrient availability have been, however, seldom included in studies of plant-mediated herbivore interactions. We hypothesized that nitrate, as one of the most important macroelements required by plants, can influence the interactions between shoot- and root-feeding herbivores. We used *Arabidopsis thaliana* plants grown under low or 33% higher nitrate availability to test the impact of aphid presence on nematodes and *vice versa* under different fertilization and furthermore analysed plant characteristics like growth and primary metabolite profiles. Aphids and nematodes had different effects on each other and these interactions were strongly shaped by plant fertilization. The results demonstrate the complexity of plant-mediated herbivore interactions and that already small alternations of abiotic conditions can be of tremendous consequence for their outcome.

POSTER 76 – SESSION 2.4

Agrobacterium rhizogenes-induced composite plants: A tool to investigat ectomycorrhizal symbiosis.

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We want to unravel signalling chains controling developmental processes and physiological adaptations associated with ectomycorrhiza formation in the plant model poplar. By using genome-wide gene expression analysis we have identified mycorrhiza-specific induced plant genes and want to use them to identify elements (promotor elements and transcription factors) responsible for regulation of mycorrhiza-based plant processes. One approach to identify cis elements in the promotor region of respective genes is the generation of stable poplar transformants containing a series of truncated promotor fragments controlling the expression of a visual marker gene. However, formation of transgenic poplar plants (*Popolus tremula x alba*) is an extremly time consuming process, which last for about 1.5 years. Due to this, we established composite poplar plants composed of transgenic roots and non-transgenic shoots. This approach is much faster and allows transgene investigtions already after about 3 month. Six different *Agrobacterium rhizogenes* strains were analyzed for their ability to form composite poplar plants harboring transgenic roots. To increase the sensitivity of visual marker detection in plant cells, we used an enhanced yellow fluorescent protein containing a peroxysomal targeting signal. First results of this novel strategy for visualization of gene expression in poplar ectomycorrhizas will be presented.

POSTER 77 – SESSION 2.4

A new approach to study the effect of arbuscular mycorrhiza in the field

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Plant communities are assumed to be shaped by competition of individuals for available resources. The concept is challenged by arbuscular mycorrhizal fungi (AMF) who build an underground network allowing exchange of nutrients and signals between many different plants of the same and different species. A thorough analysis of the importance of AMF for the plant's fitness in the field has been hampered by the lack of suitable experimental systems. Here, we conducted field experiments in the plant's native habitat in Utah, USA, with three independently stably transformed *Nicotiana attenuata* plants impaired in the formation of AMF due to silencing of calcium calmodulin-dependent protein kinase (CCaMK), the key regulator for the formation of endosymbiosis (Singh & Parniske, 2012). Stalk lengths of the transgenic lines initially tended to be shorter than for empty vector (EV) plants, but at the end of the experiments all plants had the same size and a similar root and shoot biomass. Microscopic observations of stained roots showed many fungal infection structures in EV plants and only few hyphae in the transgenic lines. Currently, the AMF communities of EV plants and of one of the transgenic lines are characterized. The results will be the basis of future field experiments to address the fundamental ecological question how AMF infection affects the plants' capacity to survive and reproduce.

POSTER 78 – Session 2.4

General and plant species-specific foliar metabolic responses to arbuscular mycorrhiza

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Arbuscular mycorrhizal (AM) fungi are commonly associated with higher land plants and regarded to be integrative parts of the plant's physiology. Mycorrhizal effects on plant chemistry have been mainly analyzed at the root level, while little is known about how the shoot metabolome responds to the symbiotic association. The improved nutrient supply and fungal carbon sink activity may affect resource allocation and thus influence plant primary and secondary metabolites at the whole plant level. Hence, in a species-comparison approach, we assessed foliar metabolic changes of five herbaceous plant species with different phylogenetic relationships using comparative metabolomics to evaluate the specificity of plant responses to AM fungi. *Rhizophagus intraradices* intensely colonized the roots of all plant species at comparable levels. AM increased shoot phosphorus levels in all plant species, whereas carbon and nitrogen contents were not affected. Using metabolite profiling and metabolic fingerprinting, we found sets of metabolites changed along with mycorrhization commonly in all plant species, which can be considered as mycorrhizal plant biomarkers, as well as metabolites specifically in- or decreased only in certain plant species. These metabolic changes should have important consequences for the ecological outcome of plant-herbivore and plant-pathogen interactions.

POSTER 79 – SESSION 2.5

Regulation of Agrobacterial *lpt* Oncogene Expression in Host plants

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In nature several economically important plant species such as walnut or grapevine become transformed with T-DNA by virulent *A. tumefaciens* strains and suffer from the crown gall disease. Genes responsible for this tumor disease are the three T-DNA-encoded oncogenes, *iaaM*, *iaaH*, and *lpt*. The aim of our studies is to unravel the molecular mechanism regulating expression of oncogenes in host cells. Our studies demonstrate that the *lpt* promoter has a eukaryotic sequence structure comprising AuxREs and W-boxes for binding of plant transcription factors. We identified two candidates from the WRKY and ARF transcription factor family which show elevated gene expression in tumors and activate the *lpt* promoter. Tumors of *wrky* mutant plants are smaller than those of the wild type, suggesting a role of WRKY in tumor development. WRKY protein binds to the *lpt* promoter *in vitro* and interacts with ARF in the plant cell nucleus. In the presence of auxin both transcription factors synergistically activate the *lpt* promoter. The activation by ARF alone is inhibited by Aux/IAA. In our model we suggest that elevated auxin levels in T-DNA transformed *Arabidopsis* cells induce degradation of Aux/IAA. Aux/IAA degradation releases ARF to form a complex with WRKY. The WRKY/ARF complex binds to the W-boxes and AuxREs in the *lpt* promoter to induce expression of the oncogene in plant host cells.

POSTER 80 – SESSION 2.6

The endophyte *Acremonium alternatum* affects plant growth and pathogen infection with clubroot

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The clubroot pathogen *Plasmodiophora brassicae* infects economically important Brassica crops such as canola and causes high yield losses. Infected plants show abnormal root growth whereas upper plant parts wither. The disease is difficult to control by chemical and cultural means and continues to spread around the globe. Infested fields can effectively no longer be used for cultivation of energy and oil plant canola for the next five years or more. Despite costly breeding of resistant cultivars, recent research leans towards alternative, low-impact, environmentally friendly methods to control clubroot. In a previous study *Acremonium alternatum*, an endophyte and known biological control agent in other countries, showed a promising antagonistic effect in clubroot infected Arabidopsis and Chinese cabbage. The means by which *Acremonium* controls pathogens is not known so far. In clubroot infected plants the fungus delays the development of *Plasmodiophora,* presumably by inducing resistance mechanisms of the host. We found several resistance genes to be differentially expressed in the tripartite interaction of *Plasmodiphora-Acremonium*-Arabidopsis. In addition the fungus seems to increase the abiotic stress tolerance of plants. The long-term goal is to contribute to an effective reduction of clubroot to be used in integrated pest management.

POSTER 81 – SESSION 3.1

RNA PROCESSING FACTOR 7 is involved in *nad*2 5′ processing in mitochondria of *Arabidopsis thaliana*.

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In *Arabidopsis thaliana* (*A.thaliana*) the formation of mature 5′ ends is one of the processing steps during the generation of functional mitochondrial transcripts. Up to now the importance of this frequently observed process is poorly understood. Likewise still little is known about the proteins involved in the maturation of 5′ termini. Recent studies revealed an essential role ofpentatricopeptiderepeat (PPR) proteins during this procedure. We now analyzed a P-class PPR protein, designated RNA PROCESSING FACTOR 7 (RPF7), which is required for the generation of the mature 5′ end of the *nad*2 mRNA. The corresponding gene is encoded on the lower arm of chromosome 2. RPF7 is a rather small protein consisting of only 8 PPR motives. The protein is highly conserved since in about 200 *A.thaliana* accessions only very few amino acid exchanges were identified. In accession Can-0 a premature translation stop codon is present in the last PPR motif. This termination codon as well as a T-DNA insertion in the corresponding mutant (*rpf*7-1) provokes an accumulation of larger precursor *nad*2 transcripts. Nevertheless still substantial amounts of mature *nad*2 mRNAs were generated both in *rpf*7-1 and in Can-0. This observation indicates that an additional RNA processing factor is involved in efficient *nad*2 mRNA 5′ processing.

POSTER 82 – SESSION 3.1

The *ccmC* upstream region influences the 5′ processing of *ccmC* mRNAs in mitochondria of *Arabidopsis thaliana*.

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Previous studies identified a *ccmC* transcript length polymorphism in mitochondria of *Arabidopsis thaliana* (*A.thaliana*). Sequence analyses upstream of the *ccmC* 5′ processing sites identified a region of around 60 nucleotides with substantial differences in the two accessions Columbia (Col) and C24. Accordingly two mitochondrial genotypes (C24- and Col-genotype) could be distinguished. It was further shown that RNA PROCESSING FACTOR 3 (RPF3), AT1G62930, is required for the generation of *ccmC* transcripts with 5′ ends at -484/482 in Col. In contrast, this factor is not required for the processing of *ccmC* mRNAs transcribed from the C24-genotype. Based on natural genetic variation we now used a linkage analysis to identify RPF6, which is responsible for the generation of *ccmC* transcripts with 5′ ends at -391/390 in C24. Both RPF3 and RPF6 are P-class pentatricopetide repeat (PPR) proteins consisting of 15 PPRs with high similarity to each other. Binding site predictions indicate that both proteins interact with the RNA upstream of the *ccmC* 5′ processing sites, in the region that is different in Col and C24. The investigation of about 50 *A.thaliana* accessions revealed additional accessions with defects in the *ccmC* mRNA processing.

POSTER 83 – SESSION 3.1

Identification of *cis*-regulatory elements for bundle sheath specific gene expression in the promoter of the *Arabidopsis thaliana* sulfate transporter SULTR2;2

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We are interested in analyzing the gene regulatory networks of mesophyll and bundle sheath cells of C3 and C4 plants. The gene coding for SULTR2;2 is one of 14 genes of the Arabidopsis sulfate transporter gene family. The promoter is active only in the phloem of the roots and the bundle sheath cells of the leaves, where the transporter transfers sulfate from the xylem in the bundle sheath cells. By means of analysis of the SULTR2;2 promoter we intend to identify *cis*-regulatory elements responsible for gene expression in the bundle sheath cells of *A. thaliana*. GUS reporter gene assays showed that it is active specifically in the bundle sheath cells of *A. thalian*a as well as in the bundle sheath of the C4 species *Flaveria bidentis*, demonstrating the conservation of the respective *cis*-regulatory elements between rosids and asterids. With the dissection of the promoter in five regions and stepwise deletion of these regions we have identified a 741 bp region that comprises elements for the cell specificity and overall activity of the promoter. The SULTR2;2 promoter of *A. thaliana* is a good starting point to explore the molecular and evolutionary aspects of tissue specific gene expression in leaves of C3 and C4 species.

POSTER 84 – SESSION 3.1

Approach to precision-engineering of plant minichromosomes

Michael Florian Mette[1], Chee How Teo[1], Eszter Kapusi[1], Lu Ma[1], Götz Hensel[1], Axel Himmelbach[1], Jochen Kumlehn[1], Ingo Schubert[1], Andreas Houben[1], Inna Lermontova[1]

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Custom-designed minichromosomes are highly desirable tools for the analysis of plant chromosome function and for plant biotechnology [1]. We have established protocols to generate functional *de novo* centromeres *via* targeting of recombinant kinetochore proteins to tandem repeat arrays at non-centromeric positions [2] as well as stably transmissible random-truncated chromosomes *via* T-DNA-mediated telomere seeding [3;4]. Now, we aim to combine these methods with TALEN-mediated targeting of chromosome truncation to engineer minichromosomes with high precision in the model *Arabidopsis thaliana* and in barley as a crop. Further, the requisites for efficient *de novo* centromere formation will be analysed and mitotic and meiotic activity of *de novo* centromeres be determined.

[1] Houben, Mette, Teo, Lermontova, Schubert (2013) Engineered plant minichromosomes. *Int. J. Dev. Biol.* in press

[2] Teo, Lermontova, Houben, Mette, Schubert (2013) *De novo* generation of plant centromeres at tandem repeats. *Chromosoma* 122:233-241

[3] Kapusi, Ma, Teo, Hensel, Himmelbach, Schubert, Mette, Kumlehn, Houben (2012) Telomere-mediated truncation of barley chromosomes. *Chromosoma* 121:181-190

[4] Teo, Ma, Kapusi, Hensel, Kumlehn, Schubert, Houben, Mette (2011) Induction of telomere-mediated chromosomal truncation and stability of truncated chromosomes in *Arabidopsis thaliana*. *Plant J.* 68:28-39

POSTER 85 – Session 3.2

Arabidopsis BPC6 interacts with LHP1 at PRE-like GAGA DNA-motifs

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The DNA-binding proteins Pleiohomeotic (PHO) and the GAGA-motif binding Trithorax-like (Trl) or Pipsqueak (Psq) are important regulators in Drosophila. They control gene expression via Polycomb repressive complex (PRC) 1 and 2 function at Polycomb repressive DNA-elements (PREs) in a context dependent manner. The equivalent DNA-binding proteins in plants that communicate with either PRC1 or PCR2 are still elusive. Here we show that *Arabidopsis thaliana* BPC6 interacts with plant PRC1 component LHP1 in the nucleoplasm. BPC6 and its paralog BPC4 are group II BPC proteins that are both characterized by an Alanine-zipper dimerization domain, which is also required for the interaction with LHP1. This BPC6-LHP1 complex functions probably in a larger association with other proteins that include PRC2 components such as VRN2 and possibly MSI1. By DPI-R-ELISA we found that BPC6 is sufficient to recruit LHP1 to GAGA-motif containing DNA-motifs. *Lhp1-4 bpc4 bpc6* triple mutants display pleiotropic phenotypes, extreme dwarfism and early flowering, which suggests synergistic interaction between LHP1 and group II BPCs. Transcriptome analyses support a synergistic function of plant GAGA-factors and LHP1 to repress homeotic genes, such as *FT*, *AGAMOUS* or *SHATTERPROOF*. Our findings suggest the requirement of group II BPCs for the recruitment of PRC1 and PRC2 components to PRE-like GAGA-motifs.

POSTER 86 – SESSION 3.2

RACK1 scaffold proteins regulate miRNA abundance in Arabidopsis

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Plant microRNAs (miRNAs) regulate many aspects of plant development including hormone responses, floral development and phyllotaxy. Biologically active, mature miRNAs associated with an ARGONAUTE (AGO) protein to bind and regulate target mRNAs. These mature miRNAs are released from longer primary miRNAs (pri-miRNA) by the RNAse III-like enzyme DICER-LIKE 1 (DCL1). Additional proteins act in concert with DCL1 (e.g. HYL1 and SERRATE) and AGO1 (SQN, HSP90) to facilitate efficient and precise pri-miRNA processing and loading into the effector protein, respectively. In order to identify novel components of the miRNA pathway we conducted a yeast two-hybrid screen using SERRATE as bait. Here, we show that the scaffold protein RECEPTOR FOR ACTIVATED C-KINASE (RACK1), one of the novel identified interaction partners, regulate miRNA abundance via several distinct mechanisms.

POSTER 87 – Session 3.3

Identification of Golgi-localized PAPS transporter in *Arabidopsis thaliana*

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Sulfations of plant hormone-like peptides PSK, RGFs and PSYs require the universal sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to be transported from the cytosol into the Golgi. Arabidopsis genomes encode four putative Golgi localised PAPS transporters- AtgPAPST1 AtgPAPST2, AtgPAPST3 and AtgPAPST4. AtgPAPSTs belong to the nucleotide sugar transporter (NST) protein family and contain 10 putative transmembrane domains. Notably, these are closer related to their homologues in vertebrate and *C. elegans* than to other NST genes from Arabidopsis. We have isolated all four *atgpapst* T-DNA insertion homozygous single knockout mutant lines and showed the subcellular localization of the same putative transporters in Golgi. As single mutants reveal no obvious phenotype the multiple knockout mutants and amiRNA plants are currently being generated. The putative AtgPAPST transporters will be heterologously expressed in yeast or *E.coli* cells and subsequenty reconstituted into artifcial systems for further characterisation of their transport properties. Furthermore, a method for quantification of sulfated peptides shall be established using peptidomics to determine whether the sulfation profile is impaired in these mutants.

POSTER 88 – SESSION 3.3

Influence of tomato subtilase 3 (SBT3) propeptide on folding and inhibition of its mature enzyme.

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Subtilases are ubiquitous serine peptidases found in all kingdoms. In plants they are involved in many processes like protein turnover, reaction to biotic and abiotic stresses, plant development, modification of the cell wall, and cell death. As extracellular proteases, they are synthesized as prepro-enzymes and targeted to the secretory pathway, where zymogen maturation occurs. We found that the prodomain of tomato subtilase 3 (SBT3) is required for passage through the secretory pathway and enzyme maturation. SBT3 mutants impaired in activity, folding or stability accumulated within the cell as unprocessed zymogens. Intracellular accumulation was also observed for a SBT3 mutant lacking the prodomain. Secretion of the mature enzyme into the apoplast was restored, when the prodomain was co-expressed with the prodomain-deficient mutant in trans. The activity of SBT3 was found to be inhibited by its prodomain, forming a stable complex with the mature enzyme. The data indicate that the prodomain assists in protein folding and, at the same time, acts as an inhibitor of enzyme activity. With respect to its function *in vivo*, we found that cell walls of transgenic SBT3 overexpressing and RNAi lines had the same overall sugar composition but differed in the degree of pectin methylesterification, highlighting a possible role of SBT3 in the interaction with cell wall modifying enzymes.

POSTER 89 – SESSION 3.3

Analysis of AtSUC4 targeting motives

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The sucrose transporter family of *Arabidopsis thaliana* consists of nine members. Analyses of the subcellular localisation of AtSUCs showed that only one member of this family, AtSUC4, localises to the tonoplast, while all other AtSUCs are plasma membrane transporters. It is known that targeting of AtSUC4 to the vacuolar membrane is mediated by Adaptor Protein (AP) complex 3. If AP-3 is missing, AtSUC4-GFP fusion proteins are no longer detected in the tonoplast, but remain in the *cis*-Golgi (Wolfenstetter *et al*., 2012). So far, AtSUC4 is the only known cargo of AP-3 in Arabidopsis. Up to now several targeting motifs for tonoplast sorting have been identified, for example di-leucine or tyrosine-based motives. AtSUC4 does not contain any of the known motifs. To find the targeting motif of AtSUC4 chimeric proteins were generated in which parts of the large cytosolic domains of AtSUC4 (N-terminus and middle loop) were exchanged for the corresponding parts of plasma membrane localised AtSUC2. Analyses of the subcellular localisation of these chimeric proteins revealed that mutations in both N-terminus and loop of AtSUC4 are essential to target this protein to the plasma membrane.

POSTER 90 – SESSION 3.3

Sorting of membrane proteins between plasma membrane and tonoplast

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The tonoplast (TP) and the plasma membrane (PM) are two endpoints of membrane traffic along the plant secretory pathway. Correct targeting of membrane proteins is essential for the generation and maintenance of membrane identity and integrity and proper cell function, cell division, and growth. Though the general trafficking mechanisms seem conserved in eukaryotes, the endomembrane system of higher plants displays distinct organizational features that may entail adaptive specializations in membrane trafficking. As limited information is available on structural determinants required for targeting of plant membrane proteins, we used the five differentially localized members of the PTR/NRT1 subfamily II from *Arabidopsis* to identify signals required for their specific targeting to the PM and TP, respectively. We found that the dileucine motif [D/E]X3-5L[L/I] at the hydrophilic N-terminus of TP-localized AtPTRs was necessary and sufficient for retargeting PM-localized PTRs and *Arabidopsis* sucrose transporter SUC2 to the TP, whereas the predicted large central loop region adjacent to the transmembrane domain 7 of the PM-localized AtPTR1 and AtPTR5 was required for targeting to the PM. Furthermore, we started to dissect intracellular trafficking routes of AtPTRs by transient co-expression with dominant negative mutants of Rab GTPases in tobacco epidermis cells.

POSTER 91 – SESSION 3.3

Regulation of *Arabidopsis* PIP5K2 by phosphorylation

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Phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) is a regulatory phospholipid with a diverse set of physiological functions. PtdIns(4,5)P2 is formed by PI4P 5-kinases and it has been shown for various eukaryotic models, including Arabidopsis, that PI4P 5-kinases can be regulated by phosphorylation. However, for plant PI4P 5-kinases only little information is available and it is unclear whether Arabidopsis PI4P 5-kinases can be phosphorylated, what amino acids are phosphorylated, and what consequences arise for localization and functionality of the enzymes. Here we investigate the phosphorylation of one Arabidopsis PI4P 5-kinase isoform, PIP5K2. Recombinantly expressed PIP5K2 was phosphorylated *in vitro* by PKA and also by complex Arabidopsis extracts containing protein kinase activity. Furthermore, phosphorylation of PIP5K2 decreased catalytic activity in an ATP dependant manner. Phosphorylation sites of PIP5K2 were determined by computer aided prediction, MS and by peptide-array analysis. Respective amino acids were changed to alanine or aspartate residues, mimicking dephosphorylated or phosphorylated states, respectively. Recombinant variant proteins were tested *in vitro* for catalytic function and subcellular localization. Variant proteins were also expressed in relevant Arabidopsis mutant backgrounds and analysed for complementation of phenotypes.

POSTER 92 – SESSION 3.3

Drought stress response in the transcriptome of European silver fir (*Abies alba* Mill.)

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Understanding the processes of adaptation to environmental change is a challenging task, especially for non-model organisms. With the advent of next generation sequencing technology, the development of molecular resources to study these processes became possible. Nevertheless, it is a challenge to find traits and genes which are involved in adaptive processes. Drought stress was identified as one of the main challenges for many forest tree species, such as *Abies alba*, the European silver fir. We present here an approach to detect genes of ecological relevance by differential gene expression profiling in a drought stress element on *A. alba* seedlings. The plants were monitored for their water status with a novel setup of terahertz time-domain spectroscopy. Gene expression profiling was performed on a pool of stressed plants vs. one of control plants using massive analysis of cDNA ends (MACE). The two pools exhibited a high number of significantly differentially expressed genes. Gene ontology analysis revealed that a large number of genes related to metabolism and photosynthesis were down-regulated, while many genes involved in stress response were up-regulated in the stressed plants.

POSTER 93 – SESSION 3.3

Post-translational protein import into the plant endoplasmic reticulum

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Chaperone assisted sorting of post-translationally imported proteins into chloroplasts, mitochondria and the endoplasmic reticulum (ER) is a general mechanism among all eukaryotic organisms. Preproteins translated in the cytosol are prone to aggregation due to molecular crowding or premature folding events. Therefore distinct molecular chaperones assist preproteins during the targeting process and help to mediate first interactions with the organellar membranes. Specific docking proteins containing tetratricopeptide repeat (TPR) domains are universally found among all cellular compartments as components of the translocon machineries. Recently, we have characterized AtTPR7 as a novel tail-anchored ER protein in plants. AtTPR7 specifically interacts with all cytosolic HSP90 isoforms as well as HSP70 via its TPR domain. Moreover, it is part of the Sec translocon and can functionally complement a Δ*sec71* yeast mutant which is impaired in post-translational protein transport. We therefore propose that AtTPR7 is involved in post-translational import into the ER in plants. Biochemical and biophysical analyses of chaperone interactions with TPR docking proteins further point to a differential recognition of HSP70 and HSP90 isoforms by the docking proteins of the ER (AtTPR7), chloroplasts (Toc64) and mitochondria (OM64).

POSTER 94 – SESSION 3.3

Functional characterization of multi-domain Sec14 proteins in *Arabidopsis thaliana*

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Sec14 proteins comprise a large superfamily of regulatory proteins at the interface of membrane trafficking and lipid homeostasis. Our recent work suggests that yeast Sec14 renders PtdIns vulnerable to PtdIns 4-OH kinase attack during PtdCho-dependent heterotypic phospholipid exchange. The resulting pool of PtdIns(4)P regulates the recruitment and activation of regulatory proteins at *trans*-Golgi membranes and is critical for the formation of secretory vesicles. Among the 31 Sec14 homologues encoded in the Arabidopsis genome, 11 comprise multi-domain proteins in which the N-terminal Sec14 domain is linked to a C-terminal NOD domain. Our current studies focus on AtSFH1, a member of the Sec14-NOD subfamily that plays an essential role in the establishment of polarized membrane trafficking in root hairs. We are particularly interested on the characterization of the C-terminal NOD domain. We were able to show that this module is required for plasma membrane targeting of AtSFH1 and will present evidence that this is dependent on a polybasic stretch that confers high specificity towards designated plasma membrane lipids. Our results also suggest that the NOD domain confers additional roles independent of plasma membrane binding and that its structural integrity is essential for AtSFH1 function. In addition, we identified suppressor mutants in a genome-wide EMS-mutagenesis screen that are able to bypass the AtSFH1 requirement for proper root hair development.

POSTER 95 – SESSION 3.4

Functions of two putative calcium channels NtTPC1 & NtMCA2 in tobacco BY-2 cells

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Calcium is involved in nearly all aspects of plant growth and development and has been firmly established as a central player in regulating numerous processes in plants. Transient elevations in cytosolic free calcium level have been documented to be involved in responses to both external and internal stimuli. This heterogeneity of increases in cytosolic-free calcium ion concentration in terms of duration, frequency, and spatial distribution is defined as calcium signatures. The intriguing question is how does specificity of response derive from such an apparently universal mechanism? One of the determining factors is the generation of such stimuli specific calcium signature which relies on the coordinated work of many calcium channels. Two genes encoding putative calcium channels have been indentified in *Nicotiana tabacum*. One is NtTPC1A/B and the other is NtMCA1/2. The former one encodes a putative voltage-gated calcium channel located at the tonoplast and the latter one encodes a putative mechanosensitive calcium channel located at the plasma membrane. Over expressing those two calcium channels to find out more information about their physiological functions and gain insights into their roles in contributing to stimuli specific calcium signature under both developmental and environmental contexts is the subject of investigation.

POSTER 96 – SESSION 3.4

Phosphorylation of an *Arabidopsis* PI4P 5-kinase involved in polar tip growth

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The regulatory phospholipid, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2), is involved in the regulation polar tip growth of pollen tubes. In plants PtdIns(4,5)P2 is synthesized by PI4P 5-kinases. A key enzyme of PtdIns(4,5)P2 formation in pollen tubes of *Arabidopsis thaliana* is PIP5K6. In different eukaryotic cells PI4P 5-kinase activity can be modulated by reversible phosphorylation. *In vitro* tests demonstrated that recombinant PIP5K6 could be phosphorylated by protein kinase A or by pollen tube extracts harboring protein kinase activity. The identification of protein kinases involved in PIP5K6 phosphorylation was carried out by an approach combining in-gel kinase assays and mass-spectrometric protein analysis. Our tests revealed several candidate protein kinases from pollen tube extract capable of phosphorylating PIP5K6. In this study we report the identification of a protein kinase that may be important for the regulation of PIP5K6. A phosphothreonine that is located in a predicted protein kinase recognition motif was identified by mass-spectrometric analysis. *In vitro*-activity tests and experiments on transiently transformed pollen tubes provided data about the influence of the substitutions on PIP5K6 functionality. Additional yeast two hybrid studies were carried out to confirm the interaction between these two proteins.

POSTER 97 – SESSION 3.4

Cell division and morphological patterning of Marchantia analysed by live-cell microscopy of GFP-labelled microtubules

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Phylogenetic analyses indicate that liverworts are the closest living relatives of the first land plants. We are interested in understanding how morphological patterning arose in the earliest land plants and how this is connected to the evolution of the cytoskeleton. The liverwort Marchantia polymorpha carries a number of important cytoskeletal novelties: it is known to exhibit preprophase bands and at the same time it shows so-called polar organizers, a specialized type of MTOC only known from liverworts. We investigated Marchantia tubulinß genes in order to establish a FP-based microtubule marker. Whereas tubulinß genes 1-3 were expressed in all tissues analyzed, tubulin ß4 was found to be male and antheridiophore specific. Expression of the GFP-Marchantia tubulinß 1 fusion by the EF1a promoter allowed live-cell imaging of various Marchantia cell types including the free swimming spermatozoids. Studies of microtubule dynamics and comparison with data from Arabidopsis suggested similar properties of interphase microtubule cortial arrays concerning self-organization. Analyses of cell division revealed the dynamic behaviour of the polar organizers and preprophase bands. To test whether microtubules control tissue patterning in Marchantia we applied microtubule drugs. The data suggest that microtubules, likely by the aid of the preprophase band, direct division plane positioning in Marchantia. The bearings of these results for the mechanism of morphological patterning of early land plants is discussed.

POSTER 98 – SESSION 3.4

Putative ROP-GAPs are essential for pavement cell shape establishment

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Small GTPases act as molecular switches in the signal transduction. These small proteins are divided in five subfamilies Ras, Rab, Ran, Arf and Rho GTPases. In plants - Rho GTPases are called Rho of Plants (ROPs) and regulate processes like vesicle trafficking and cytoskeleton organization. Rho proteins are inactivated by GTPase activating proteins (GAPs) by stimulating the intrinsic GTPase activity. *Arabidopsis thaliana* encodes two subfamilies of ROP-GAPs, one of which contains a Pleckstrin homology domain at their N-terminus and is therefore designated PH-GAP subfamily. PH-GAP family includes three closely related members - REN1, PH-GAP1 and PH-GAP2. REN1 was characterized as a regulator of ROP1 activity during polarized pollen tube growth. In our studies we focus on the PH-GAP1 and the PH-GAP2. Double mutants display loss of pavement cell shape complexity which is reminiscent of ROP constitutively active mutants. This observation suggested that PH-GAP1 and PH-GAP2 might regulate ROP2, ROP4 and/or ROP6 activity. In addition we could show that the GAP domain is essential for PH-GAP function.

POSTER 99 – SESSION 3.5

The unicellular green alga *Chlamydomonas reinhardtii* - an asocial protist?

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Programmed cell death (PCD) is a fundamental biological process, serving many important functions in development and homeostasis of multicellular organisms. Since the discovery of apoptosis in *Saccharomyces cerevisiae* [1], PCD has been also reported to occur in all protists studied so far including unicellular algae [2]. It has been suggested that PCD of protists can be regarded as an altruistic death of damaged cells to enable the survival of the particular population [3]. Therefore, we have investigated the effects of various well-known inducers of apoptosis on the unicellular green alga *Chlamydomonas reinhardtii* using the wall-deficient strain cw15. Mastoparan, H2O2 and several protease inhibitors resulted in a necrotic cell death of all cells within 2 hours, accompanied by bleaching. In these cases, the *Chlamydomonas* cells could be rescued by addition of antioxidants. Application of topoisomerase inhibitors, like camptothecin and etoposide, however, caused necrotic cell death without bleaching when the cells entered the division phase. Our findings indicate that there is no altruistic programmed cell death in the unicellular green alga *Chlamydomonas reinhardtii*.

[1] Madeo, F., Fröhlich, E., and Fröhlich, K.-U. (1997) J. Cell Biol. 139: 729-734.

[2] Deponte, M. (2008) Biochim. Biophys. Acta 1783:1396-1405.

[3] Fröhlich, K.U., and Madeo, F. (2000) FEBS Lett. 473: 6-9.

POSTER 100 – SESSION 3.5

Identification of a piece of the regulatory network that induce DUR3 and other N transporters during senescence

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Previous results indicated that during developmental and nitrogen (N) deficiency-induced senescence urea is a quantitatively important N form utilized in leaves for phloem loading and that DUR3-mediated urea retrieval contributes to the overall N retranslocation during leaf senescence (Bohner et al., under submission).

As senescence is a highly organized process we wanted to identify the regulatory network behind the induction of DUR3 as well as other N transporters known to be involved in N retranslocation during senescence. An analysis of the promoter region as well as coexpression analysis of *DUR3* and other N transporters showed that several candidate genes might act upstream of these transporter genes. These candidate genes seem to act in the salicylic acid (SA)-dependent pathway in senescence regulation. Currently, mutant lines of these genes are investigated for their phenotypic behavior during senescence as well as for changes in their expression pattern of N transporters and for their N retranslocation efficiency.

POSTER 101 – SESSION 3.5

Investigating the role of mitochondria during natural leaf senescence

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In a photosynthetically active leaf, the metabolism depends on the synergetic functions of the three energy organelles i.e. chloroplasts, peroxisomes and mitochondria. However, during the process of natural leaf senescence, chloroplasts are rapidly dismantled and as a consequence, the metabolism has to be reorganized. In tandem with peroxisomes, mitochondria thus become the main source for cellular energy, in particular necessary to support degradation processes and the subsequent reallocation of nutrients. However, mitochondria are also involved in many more metabolic processes and an extended view on mitochondrial functions during leaf senescence is still missing. Here, we first report on our efforts to gain more insights into the role of mitochondria during natural leaf senescence by combining transcriptomic, metabolomic and physiological approaches. Secondly, in order to identify novel and senescence-specific mitochondrial functions, we generated from publicly available arrays a manually curated list of genes encoding for mitochondria-targeted proteins. We are currently investigating the functions of a set of genes of unknown function by analyzing T-DNA insertion lines for senescence specific phenotypes and challenging them at a physiological, biochemical and molecular level. Taken together, these two approaches provide us with new insights into the mitochondrial functions involved in the regulation of metabolism in response to natural leaf senescence.

POSTER 102 – SESSION 4.2

THE MECHANISM OF ACTION OF STRIGOLACTONE

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Plants are sessile and therefore their development needs to be environmentally responsive. One such plastic developmental process is shoot branching, where axillary meristems are produced which may remain dormant or active to produce a branch. The phytohormone auxin has been proposed to mediate this aspect of development by its transport. One hypothesis is that axillary meristem activity depends on the canalization of auxin transport away from the axillary meristem and out into the main stem. Auxin transport canalization involves a feedback mechanism whereby an initial flux of auxin from a source to a sink upregulates the production and polarization of its own efflux transport proteins (eg. PINs), establishing files of cells actively transporting auxin from the source to the sink. A computational model based on this mechanism can reproduce a range of branching phenomena. In this model, diverse effects of a second hormone, strigolactone (SL), can be reproduced if SL acts to promote PIN removal from the plasma membrane. This role of strigolactone was supported by experimental results in which strigolactone treatment triggered PIN1 depletion from the basal plasma membrane in xylem parenchyma cells. This removal is independent of protein synthesis but dependent on clathrin-mediated endocytosis.

POSTER 103 – SESSION 4.2

Counting the number of stem cells in the shoot apical meristem of Arabidopsis thaliana

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Question: All aerial tissue of A. thaliana is formed from the pool of stem cells located on the growing tip of the plant, the shoot apex. The only known marker gene for these stem cells is CLAVATA3, encoding a small signalling peptide. CLV3 is expressed in more than 30 cells, however there is indication that not all of them contribute to plant growth in the long run. We therefore want to determine how many true long-term stem cells are present in the shoot apical meristem of A. thaliana. Methods: Plants are transformed with a Brainbow-style fluorescent reporter construct. Upon induction of CRE recombinase activity in the CLV3 domain, individual cells are irreversibly switched from the default GFP expression to either RFP or BFP. The descendants of these switched cells form clonal sectors of a certain colour. The relative size of this sector allows to infer the number of stem cells. If for example a sector covers a third of the stem cross section, most likely three stem cells are present. Results and Conclusions: Preliminary experiments indicate that there are two to four true long-term stem cells in each of the three meristem layers, making a total of six to twelve stem cells. This is far less than the number of CLV3 expressing cells, suggesting that there are at least two different subpopulations of cells in the CLV3 domain.

POSTER 104 – Session 4.2

CLE peptide signaling inhibits cambium activity in *Arabidopsis* in a MOL1-dependent fashion

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Question: We recently identified the leucine-rich repeat receptor-like kinase (LRR-RLK) MORE LATERAL GROWTH 1 (MOL1) that inhibits cambium activity in *Arabidopsis*. MOL1 has a putative CLE-peptide binding site with high similarity to that in CLV1, an LRR-RLK active in shoot apical meristems. In this study we aim to identify the putative ligand of the MOL1 receptor and elucidate the role of MOL1 in cambium regulation. Methods and Results: *MOL1* promoter-reporters show that *MOL1* is expressed in phloem cells abaxially to the cambium. We tested candidate CLE peptides for their cambium regulating activity and found that one CLE peptide specifically reduces cambium activity in a *MOL1*-dependent manner. Interestingly, promoter-swapping experiments show that *MOL1* can partially replace *CLV1* in the shoot apical meristem. Furthermore, *CLE41*, which promotes cambium activity and is expressed in the phloem, is more active in *mol1* mutants. Conclusions: We conclude that *MOL1* acts in the cambium in a similar fashion as *CLV1* in shoot apical meristems. Like *CLV1*, *MOL1* represses meristem activity depending on the activity of a CLE signaling peptide. We propose that the non-cell autonomous effect on cambium activity downstream of *MOL1* depends on reducing the levels of mobile CLE41 peptide in the phloem. Collectively, our analyses reveal a strong similarity in the regulation of apical and lateral plant meristems.

POSTER 105 – Session 4.2

cpk28 loss-of-function mutant has a developmental phenotype associated with the activation and repression of the jasmonate and gibberellic acid signaling pathways

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Calcium dependent protein kinases (CDPKs) are highly conserved serine/threonine kinases translating a calcium signature into phosphorylation of a substrate resulting in downstream adaptive responses. CDPKs were predominantly characterized in rapid plant abiotic stress and immune signaling responses, with only little focus laid on CDPK function in plant development. Here we report CPK28 in Arabidopsis as regulator of shoot elongation and vascular development. Only upon the transition to the generative growth phase, *cpk28* mutant plants displayed shorter stems as well as increased secondary growth. Furthermore, *cpk28* mutant plants have shortened petioles, showing enhanced anthocyanin production. While the stem elongation phenotype correlated with reduced transcript levels of GA (gibberellic acid)-biosynthesis genes, it could be partially rescued by exogenous application of GA. The competence to form the *cpk28* mutant phenotype is dependent on the presence of a functional jasmonate biosynthesis and signaling pathway. Consistently we observed an elevated jasmonic acid (JA) content and JA marker gene expression in *cpk28* mutant plants. However *cpk28* mutant plants did not show an increases resistance to the fungal pathogen *Alternaria bassicicola* and the herbivores *Trichoplusia ni* and *Spodoptera littoralis* suggesting a role for CPK28 in developmental processes involving the phytohormones GA and JA rather than in defense signaling.

POSTER 106 – Session 4.2

Role of the bHLH transcription factor HECATE1 in the stem cell network of *Arabidopsis thaliana*

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Higher plants keep the ability to generate new organs throughout their life cycle. This remarkable characteristic relies on their capacity to maintain pools of stem cells, which are continuously dividing and differentiating. In *Arabidopsis thaliana* apices, stem cells are embedded in a microenvironment called the Shoot Apical Meristem (SAM). Establishment and maintenance of this structure require the activity of the *WUSCHEL/CLAVATA3* (*WUS/CLV3*) feedback loop. We recently identified the basic Helix-Loop-Helix (bHLH) transcription factor HECATE1 (HEC1) as a direct WUS target and showed that it regulates stem cell fate in a cell-type specific manner. Strikingly, its ectopic expression in the stem cell domain (Central Zone, CZ) drives stem cell overproliferation partially independently of the WUS/CLV3 pathway. Furthermore, *HEC1* misexpression in the *WUS* domain (Organizing center, OC) impairs stem cell maintenance. Using genome-wide transcriptional profiling, we are currently investigating the mechanisms underlying HEC1 domain-specific activity. We recently found that HEC1 positively regulates *ARR7*, which is a negative regulator of *WUS* expression and cytokinin signaling. Our current model positions *HEC1* as part of an additional loop of communication between the CZ and the OC through the movement of ARR7.

POSTER 107 – SESSION 4.2

Cell-to-cell movement of a stem cell inducing transcription factor in *Arabidopsis thaliana*

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The shoot apical meristem (SAM) gives rise to all above ground tissues of a plant and while the appearance of a plant changes dramatically throughout its life, the structure of the meristem remains remarkably constant. This is achieved by a tightly regulated network in which the homeodomain transcription factor WUSCHEL (WUS) plays a key role. Plants mutant for *wus* are unable to constantly maintain stem cells resulting in an arrest of growth and infertility1. It has been shown that although WUS is expressed only in deeper layers of the SAM it can move into the overlying stem cells, suggesting a direct function of WUS in these cells2. We try to understand the mechanism and dynamics of WUS movement and we are developing a system to interfere with WUS protein function in a cell specific fashion to determine whether the observed movement of WUS is functionally relevant.

1 Laux et al. Development, 1996; 2 Yadav et al. Genes & Development, 2011

POSTER 108 – SESSION 4.3

The role of heat stress transcription factor HsfA2 in vegetative and reproductive tissue development and heat stress response in tomato (*Solanum lycopersicum*)

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Heat stress transcription factors (HSFs) trigger the transcriptional activation and rapid accumulation of heat shock proteins to compensate for the disturbed proteostasis under adverse stress conditions. Tomato genome comprises 24 HSF genes but major aspects of heat stress response (HSR) and recovery are regulated by the interaction of HsfA1a with HsfA2 and HsfB1. Using a bioinformatics co-expression approach we identified a putative regulatory network for HsfA2 with potential role in abiotic stress responses. HsfA2 is expressed at basal levels in vegetative tissues under non-stress conditions but is strongly induced in early stages of pollen development indicating possible developmental function. To investigate the role of HsfA2 in plant development and HSR, we used tomato lines (*S. lycopersicum* cv Moneymaker) transformed with expression cassettes encoding HsfA2 in sense and antisense orientation either causing its constitutive expression or RNAi-mediated knock-down, respectively. Phenotypic analyses showed that HsfA2 is involved in several developmental aspects including pollen quality. To get more insights into the role of HsfA2 in both vegetative and reproductive tissues, we performed a transcriptome analysis using Next Generation Sequencing of control and heat stressed leaves and anthers. Our results show major differences between the HSR in vegetative and male reproductive tissues and point to the direction of HsfA2 as an important transcriptional regulator of many genes related with thermotolerance.

POSTER 109 – Session 4.3

The plastid-localized NAD-dependent malate dehydrogenase is crucial for energy homeostasis in developing *Arabidopsis thaliana* seeds

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In the absence of photosynthesis, ATP is imported into chloroplasts and non-green plastids by ATP/ADP transporters or formed during glycolysis, the latter requiring continuous regeneration of NAD+, supplied by the plastidial isoform of NAD-MDH. During analysis of T-DNA insertion mutants of *A. thaliana* only heterozygous but no homozygous mutants could be identified. These heterozygous plants show higher transcript levels of an alternative NAD+-regenerating enzyme, NADH-GOGAT, and, remarkably, improved growth when ammonium is the sole N-source. In-situ hybridization and GUS-histochemical staining revealed that plNAD-MDH was particularly abundant in male and female gametophytes. A knockout of plNAD-MDH has a strong effect on pollen tube growth. Knock-out pollen lacking plNAD-MDH do not germinate in vitro, but can fertilize the egg cell in vivo. However, young siliques of selfed heterozygous plants contain both green and white seeds corresponding to wild-type/heterozygous (green) and homozygous knock-out (white) mutants in a (1:2):1 ratio. Embryos of the knock-out seeds only reached the globular stage, did not green, and developed to tiny wrinkled seeds, suggesting that a blocked major physiological process in *plNAD-MDH* mutants stops both, embryo and endosperm development in order to avoid assimilate investment in compromised offspring.

POSTER 110 – SESSION 4.3

Ethylene regulates development and composition of seeds

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Reproduction in flowering plants critically relies on a complex fertilization process involving synergid-mediated pollen tube attraction, degeneration of the first synergid, and gamete fusion. Successful double fertilization triggers programmed cell death of the second synergid, thereby contributing to the establishment of a pollen tube block. We previously showed that the second synergid fails to undergo PCD in ethylene hyposensitive *ein3 eil1* mutants, resulting in the attraction of supernumerary pollen tubes. Intriguingly, the persistent synergid expresses two endosperm markers and divides synchronously with the nuclei of the endosperm after fertilization, indicating that this otherwise terminally differentiating cell can be reprogrammed into endosperm. Here we address whether and how the resulting synergid-derived asexual endosperm fraction affects seed development.

POSTER 111 – SESSION 4.4

The role of the xylulose 5-phosphate/phosphate translocator (XPT) in generative and vegetative developmental processes of *Arabidopsis thaliana*

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The xylulose 5-phosphate/phosphate translocator (XPT) belongs to the plastidial phosphate translocators, which mediate the exchange of metabolites between the stroma and the cytosol across the inner envelope membrane. Previously it has been shown that the XPT is capable of transporting the C5- and C3-sugar phosphates xylulose 5-phosphate and triose phosphates (Eicks et al., 2002). The XPT might hence represent a link between the plastidial and the cytosolic branches of the pentose phosphate pathway. As a single copy gene the XPT is expressed in all major tissues. Surprisingly a T-DNA-Insertion mutant does not show any obvious phenotyp during vegetative development. However, there were profound differences during generative development compared to the wild type. Whilst the number of siliques per plant was increased, their length was decreased in the mutant. Interestingly the mutant´s seeds were about 10% bigger compared to the wild type and seedlings resulting from these seeds were also increased in size. Our data suggest that a loss of XPT function perturbes generative development at an early stage, which leads to less but bigger seeds. The basis for these developmental alterations will be the subject of future studies.

Eicks et al. (2002), Plant Physiol. 128:512-522

POSTER 112 – SESSION 4.4

Characterisation of molecular factors determining asymmetry in flowers of *Arabidopsis thaliana*

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The formation of the correct flower structure is important for the reproductive success of plants. Asymmetrical flowers - flowers which differ in shape or size from the corresponding wild type - can not only lead to a reduced, but sometimes also to higher reproductivity. To identify and characterise novel factors, which cause flower asymmetry, a single-copy *Ds* transposon insertion line of *Arabidopsis thaliana* with abnormal flowers was identified. The flowers of this mutant differ phenotypically significantly from the corresponding wild type. The mutant - hereinafter called *flower in flower* (*fif*) -shows a completely altered inflorescence and an abnormal composition and incomplete formation of floral organs. The *fif* flowers do not only lack the white coloured petals but also have stamen which are partially converted into small secondary flowers and show insufficiently fused carpels leading to a reduced seed production and disturbed germination. Initial data of the phenotypic analysis and of the molecular characterisation of this mutant line will be presented here. Our results may lead to new insights into the formation of asymmetric flowers.

POSTER 113 – SESSION 4.4

Identification of plant microProteins

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Many proteins do not function alone but are engaged in larger protein complexes. MicroProteins (miPs) are small proteins, solely consisting of a protein-protein interaction (PPI) domain. They function as negative regulators by interacting with their target proteins and preventing them from forming functional complexes. Several miPs negatively regulating the function of transcription factors have been identified in plants so far. We performed a systematic screen for small proteins with miP characteristics in the proteome of *Arabidopsis thaliana* and identified 37 new potential miPs that may be involved in processes like flowering time regulation and vasculature formation. Some of these putative miPs show high degrees of conservation in plants while others evolved more recently and can only be found dicotyledonous plants.

POSTER 114 – SESSION 9.2

A role for 14-3-3 proteins in floral transition

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We identified the transcription factor FD as a 14-3-3 interacting protein whose phosphorylated binding motif localizes to the C-terminus. FD is known to act in concert with the "florigen" FT (FLOWERING LOCUS T) to activate floral identity genes in the shoot apex and thus, to promote flowering (1, 2). Interestingly, site-directed mutation of FD abolishes the interaction not only with 14-3-3 but also with the FT. Since FT interacts weakly with 14-3-3 in an unphosphorylated manner, this suggests 14-3-3s to function as an intermolecular bridge. Remarkably, the weak 14-3-3:FT interaction is significantly increased in the presence of the phosphorylated FD-binding motif, suggesting a conformational change in FT which stabilzes the trimeric complex and might modify the DNA binding activity of FD. Accordingly, we are currently trying to reconstitute such a complex that in contrast to unphosphorylated FD should be able to bind to promoter elements in floral identity genes. Furthermore, the mutated FD version localizes to nuclear speckles, comparable to TFL1 (TERMINAL FLOWER1), which is closely related to FT but functions in an antagonistic manner.

1. Wigge PA et al. (2005) Science, 309, 1056-9

2. Abe M, et al. (2005) Science, 309, 1052-6

POSTER 115 – SESSION 4.5

Mitochondrial gamma carbonic anhydrases are required for early plant development

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NADH dehydrogenase complex (complex I) is part of the oxidative phosphorylation system (OXPHOS) in mitochondria and has an important function in energy metabolism. Unique to photoautotrophic eukaryotes such as plants and green algae is the extra gamma carbonic anhydrase (γCA) domain attached to the matrix site of the membrane arm of complex I. Altogether, three γCA (γCA1, γCA2, γCA3) and two γCA-like (γCAL1, γCAL2) proteins are known, but only three of them can compose the individual complex I γCA domain. The function of these proteins is still unclear. In earlier studies, a loss of complex I activity was reported for *γca2* mutants, but this did not come along with alterations in phenotype. Therefore, crosses between all the individual Δ*γca* mutant plants were carried out. The most significant result from these crosses was that homozygous *γca1γca2* double mutants died after opening of the cotyledons and could not been rescued so far. We hypothesize that this lethal phenotype is caused by defects in embryogenesis. In preliminary experiments siliques of plants homozygous for *γca1* and hemizygous for *γca2* were examined and embryos were dissected. In comparison to normal developing green seeds, 20 % of the examined seeds were palish green with a delayed embryogenesis. Most probably, this defect causes the aborted development of the seedling.

POSTER 116 – SESSION 4.5

Hydropriming and Osmopriming Effects on Cowpea (*Vigna unguiculata L.*): involvement of oxidative stress.

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Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance. Common priming techniques as hydropriming and osmopriming contribute to significant improvement in seed germination and seedling growth in different plant species. Production of H2O2 during the early imbibition period has been demonstrated; ROS produced after imbibition are assumed to play a role in seed germination. Thus, these reports suggest that ROS might play a signaling role in seed germination and dormancy. Although several lines of evidence indicate that ROS affect seed germination, there is little information establishing a direct link between the changes in levels of ROS anti-oxidative activities and priming. This experiment was conducted to investigate the effects of hydropriming and osmopriming on germination rate, root-shoot length and root-shoot weight of cowpea (*Vigna unguiculata*) seeds related to ROS formation (H2O2 and superoxide) and levels of glutathione and ascorbic acid . Priming was done by hydropriming with distilled water (6 hours and 2x3 hours) and osmopriming with PEG 6000 at 20 %.

Key words: Hydropriming, osmopriming, ROS, oxidative stress, seed, cowpea.

POSTER 117 – Session 4.5

Conifer embryos in shape - Interplay between genes and auxin in somatic embryogenesis of *Larix decidua*

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We are interested in the regulation of embryogenesis in conifers, in particular in the embryo shaping mediated by the meristem (SAM). Organ initiation is orchestrated by the SAM, which is the known location and target for many key regulators in angiosperms. To study the transferability to conifers we used somatic embryos of *Larix decidua* as model and experimental system. To this end we intended to identify correlations between genes with relevance to embryogenesis and hormonal control directed by auxin. We interfered into the auxin flux by applying auxin inhibitor NPA to the culture medium. NPA treated embryos failed to develop proper cotyledons, which resulted in either 'cucumber' or 'cup-shaped' morphology. The expression profile of several genes of interest (*LdLEC1, LdPIN, LdSTM, LdWOX2*) was influenced by auxin inhibition. Embryos divided into their basal and apical parts showed variations in expression according to morphology. Inhibited auxin transport has an effect on embryo formation and leads to an altered development of cotyledons and thus probably to an impact on SAM deveopment. Furthermore the resulting shifts in transcript abundance reveal a close link between hormone and gene regulation. Morphology and expression studies point towards relevant functions of the analysed genes during somatic embryogenesis of *Larix decidua* and further suggest a conserved role of principal regulators.

POSTER 118 – SESSION 5.1

Spicy selection - Differential salt avoidance machanisms for replacement evolution of invasive rose species

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The genus *Rosa* is not known for its marked salt tolerance, nevertheless *Rosa spinosissima* and *Rosa mollis* are naturally spread along the shore of the North and the Baltic Sea. Introduced from East Asia *Rosa rugosa* is also growing invasively in those habitats and replaces the native roses. Our project tries to unravel the success of *R. rugosa* with investigations in its potentially present salt avoidance mechanisms as a possible reason for the advantage over *R. spinosissima* and *R. mollis*. The major constraints of salinity, osmotic adjustment, ion toxicity, ion imbalance and photosynthesis were studied and correlated to changes in morphology. Analyses of physiological parameters were done by chlorophyll fluorescence-, fluorometric PAM- and CO2-gas exchange-measurements. Knowledge about ion uptake and transport in roots was gained with the "Pitman-chamber". At the end of the experimental period plant tissue was analysed to achieve information of the containing osmotically relevant elements and sugars. We detected, that *R. rugosa* has a higher competitive capacity at saline conditions as the other species. Data points to a species-specific regulation. Detailed information about the "how" are still lacking, but one hint for further research is, that *R. rugosa* has the lowest uptake-rates in the roots, while ion selectivity is not marked.

POSTER 119 – SESSION 5.2

Natural variation in the *Arabidopsis* circadian clockwork

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The circadian clock allows plant and animals to anticipate upcoming environmental changes and to mount an appropriately timed response. In the model plant *Arabidopsis thaliana*, several interlocked loops control the expression of ~35% genes and contribute to plant fitness. We performed a search for natural dominant modifiers of clock function by crossing a reporter construct (*TOC1:LUC*) in the Col-2 background to 100 accessions. A single accession, Lö-1, increased *TOC1:LUC* amplitude 4-5 fold over controls, while maintaining a 24-hr period and a normal phase. Other circadian reporters showed normal amplitude when crossed to Lö-1, indicating a specific effect on the *TOC1* locus. A *TOC1* promoter resection series revealed that the high amplitude phenotype was mediated by the 5′ UTR. Endogenous *TOC1* mRNA levels were not affected in F1 plants, suggesting that the Lö-1 modifier acts at the post-transcriptional level. The *TOC1* 5′ UTR contains 2 potential upstream open reading frames (uorf), one being conserved in *A. thaliana* relatives. We hypothesize that a uORF may play a role in controlling *TOC1* translation: to test this, we have generated new *TOC1:LUC* reporters whereby each uORF ATG has been mutated. The high amplitude phenotype behaves as a single semi-dominant locus, which we have mapped to ~ 30 kbp on the upper arm of chromosome 3, away from known clock genes.

POSTER 120 – SESSION 5.4

Status and Potential Use of Medicinal and Aromatic Plants in Pamir Region of Tajik and Afghan Badakhshan

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Lying at the junction of Asia's mightiest mountain ranges – the Himalayas, Karakoram, Hindu Kush, and Tine Shan – the individual valleys of the Pamir Mountains share a rich and common flora, fauna, and geography. The residents of the Pamirs, given their remoteness combined with high rates of poverty, have traditionally relied on local plants with purportedly medicinal value to treat sicknesses. Environmental, political, social, and economic developments over the past century, though, have had a negative impact on this traditional system of medicine. Thus, this study has sought to document indigenous knowledge of these medicinal plants. To this end, over the past four years, information on the previous and current status of medicinal plants and their trends in natural habitat have been collected and documented through 248 individual interviews, informal group discussions, and personal observations in the field. The collected data suggests that local residents in the Tajik-Pamirs use 92 different species of plants belonging to 34 families and 60 genera, while 31 species of plants belonging to 20 families and 27 genera are used for medicinal purposes in the Afghan-Pamirs. The study further reveals that 68% of the plants have similar uses in both the Afghan- and Tajik-Pamirs for treating illnesses, though some variations in preparation and usage of these medicinal plants were also noted.

POSTER 121 – SESSION 5.4

How did the photorespiration change in the context of C4 evolution?

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The world's most prominent enzyme ribulose bisphosphate carboxylase/oxygenase (RuBisCO) cannot just fixate CO2, it is also able to fixate O2. Result of the oxygenation reaction is the toxic molecule 2-phosphoglycolate. This molecule has to be recycled to 3-phosphoglycerate in a costly process called photorespiration. To avoid photorespiration the C4 photosynthesis evolved. This photosynthesis type is based on the suppression of RuBisCOs oxygenation reaction via an enrichment of CO2 around RuBisCO. Therefore C4 plants separate the CO2 primary fixation in the Mesophyll tissue from its final fixation in the Bundle Sheath tissue of the leaf. Along with RuBisCO most of the photorespiratory enzymes are restricted to the Bundle Sheath tissue in fully-fledged C4 plants like *Zea mays*. This work points to the question how the photorespiratory gene expression changed in the context of C4 evolution. To answer that question we use the genus *Flaveria* that has a more recent C4 origin than *Z. mays* and contains C4, C3 as well as C3/ C4 intermediate plants and is therefore useful for evolutionary studies. By using plants with this different photosynthesis types we try to firstly, analyze the changes in the localization of different photorespiratory mRNAs and secondly the changes in photorespiratory promoter activity on the example of photorespirations entry enzyme 2-phospho glycolatephosphatase.

POSTER 122 – SESSION 5.4

Homogenization of parental 35S ribosomal DNA in ancient allohexaploid *Atropa belladonna*

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The nuclear encoded, tandemly arranged rDNA represents an attractive model for investigating mechanisms of molecular evolution of repeated sequences in different taxonomic groups. Of special interest is the question of rDNA inheritance and evolution in plant hybrids, where divergent variants of 35S rDNA units donated by diploid parents coexist and evolve in polyploid genomes. Here, 35S rDNA of *Atropa belladonna* was evaluated by a combination of FISH, Southern blotting, cloning and sequencing. Ribosomal DNA repeats were evaluated in *A. belladonna*, which represents one of the oldest (10 Myr) known allohexaploid plant species originated by natural hybridization of as yet unidentified parental species. Three pairs of 35S rDNA hybridization signals were detected on separate chromosomes, but silver-staining produced only four signals per cell, indicating that two of six rDNA loci are transcriptionally inactive. In contrast, only two length variants of rDNA repeats located at different chromosomes inherited from parental species were found. The rDNA repeats demonstrate a high level, 97.2 to 99.9 %, of sequence similarity. In conclusion**,** the 35S rDNA repeats of one parent were removed and substituted by rDNA of the second parent in *A. belladonna*. In more recent hybrids it was observed that firstly one parental rDNA was inactivated; this later can lead to complete loss of a specific group of rDNA repeats.

POSTER 123 – SESSION 5.5

Spatial genetic patterns and phylogeography of *Larix decidua* (Mill.) - comparing markers with different modes of inheritance

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The genus *Larix* is distributed in the temperate and boreal biomes of the northern hemisphere. In Europe it is mainly represented by *L. decidua*, with a fragmented range in the Alps, the Sudetes, the Tatra and the Carpathian Mountains. Because of its valuable and strong wood, European Larch has been planted widely in the past and until now no phylogeographic approach exists for *L. decidua* to detect different lineages and non-autochthonous populations. Therefore, a range-wide survey of *L. decidua* populations was performed, employing markers of three genomes with contrasting modes of inheritance. Mitochondrial markers revealed a geographic pattern with two major lineages, one in the Alpine region and one in Central Europe. This was supported by the results of microsatellite nuclear markers. In contrast, chloroplast markers exhibited very low levels of variation and differentiation. Gst for mitochondrial markers was much higher than that for chloroplast markers (Gst=0.62 vs. Gst=0.03). This trend is consistent with other conifers studied so far, but the case of *L. decidua* is an extreme one. Furthermore, nuclear and mitochondrial data provided convincing evidence for human translocations in the last two centuries.

POSTER 124 – SESSION 5.6

Carbon allocation trade-off between arbuscular mycorrhizal fungi and roots reveals contrasting foraging strategies in pioneer plant species on sand

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Nutrient-poor, open sand ecosystems are of interest regarding plant nutrition and its relation to successional progress. In these systems efficient nutrient acquisition mechanisms are crucial for species success. In this regard most studies emphasize the advantages of extensive fine root systems. However, some successful pioneer species develop only coarse root systems, raising the question for alternative nutrient acquisition strategies. We hypothesize (i) that these plants allocate high proportions of C to arbuscular mycorrhizal fungi (AMF) to substitute for root surface and (ii) that nutrient depletion via AMF is equally efficient as via extensive fine root systems. To examine these hypotheses we performed a controlled experiment with five pioneer plant species: three forbs (*Plantago lanceolata*, *Hieracium pilosella*, *Hypochoeris radicata*) and two grasses (*Festuca psammophila*, *Corynephorus canescens*). In contrast to the grasses all forbs were highly dependent on mycorrhiza. Accordingly, data on root and hyphal growth confirmed contrasting strategies with predominant C-investment into AMF or roots in forbs and grasses, respectively. Soil P and N depletion rates proofed AMF as an equally efficient foraging strategy as fine root allocation. Our study emphasizes the importance of fungal parameters to clarify the relationship between species-specific traits and succession.

POSTER 125 – SESSION 6.1

Heterologous expression of putative diamine oxidases which might be involved in pyrrolizidine alkaloid biosynthesis

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Pyrrolizidine alkaloids (PA) are toxic secondary metabolites used for chemical defense in several, not closely related plant families. Factors involved in PA biosynthesis are largely unknown except of the first pathway-specific enzyme, homospermidine synthase (HSS) which catalyzes the formation of homospermidine. HSS was recruited by gene duplication of a gene involved in primary metabolism encoding deoxyhypusine synthase (DHS). For several PA producing plants, including Asteraceae, Boraginaceae, Orchidaceae and Convulvaceae, multiple independent gene duplication events of HSS have been described, but regulation and localization is highly variable. Using substracted cDNA libarys and RACE techniques it was possible to identify candidate genes encoding proteins of the diamine oxidases (DAO) family, discussed to be responsible for further procession of homospermidine. Phylogenetic analyses and gene expression studies of different plant tissues suggest two putative DAO isoforms, which might be involved in PA biosynthesis. Heterologous expression of these resulted in soluble GST fusion proteins whose biochemical characterization is currently running.

POSTER 126 – SESSION 6.1

One of a kind - *the Lycopsamine Type Pyrrolizidine Alkaloids*

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Pyrrolizidine alkaloids (PAs) are toxins produced by plants as protection against herbivores. One structural type of PAs is the lycopsamine type which is characterized by a unique necic acid. PAs of that type occur in three not closely related families, the Asteraceae (only the tribe Eupatorieae), the Boraginaceae and the Apocynaceae. Against that background it is interesting to understand the biosynthesis of this necic acid with special focus on its evolution. Has also an enzyme of primary metabolism been recruited by gene duplication as it was described for the homospermidine synthase (HSS) in the necine base biosynthesis? Earlier studies suggest that an acetohydroxyacid synthase(AHAS)-like enzyme could be involved in the first step of the necic acid pathway. Complementary substracted cDNA libraries from three different Lycopsamin-PA-producing plants resulted in the identification of AHAS-sequences putatively involved in PA-biosynthesis. Further support for this biological function will be obtained by quantitative transcript analyses and heterologous protein expression in *E. coli* for biochemical characterization.

POSTER 127 – Session 6.1

Phylogeny, ploidal variation and antimicrobial activity of *Rhododendron* (Ericaceae)

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Plants are creative 'chemists' that have evolved fascinating and biologically active chemical substances in response to microbial pathogens and herbivore pressure. In this context, polyploidy may increase the probability of evolutionary innovations (e.g., new substances). In our project, we test the hypothesis that anti-microbial activity of secondary compounds produced by the 1,300 currently accepted species of *Rhododendron* species against bacteria has a phylogenetic component and whether it is related to ploidy level. We here present a phylogenetic hypothesis based on 250 species using nuclear (ITS, *RPB2-i*) and plastid (*trn*L-F, *mat*K) DNA markers, data on antimicrobial activity of 120 species and ploidy levels of ca. 70 species. Phylogenetic analyses support the currently accepted subgenera and in most parts also sections/subsections despite incongruences between nuclear and plastid markers. Polyploids are almost exclusive to subgenera *Rhododendron* (incl. subg. *Vireya*). Regarding antimicrobial activity, the majority of species within subgenus *Rhododendron* produce substances that significantly inhibit growth of gram-positive bacteria, but activity to gram-negatives is restricted to few species in different subgenera. A novel diterpene showing the highest activity of single compounds against gram-positive bacteria has been identified.

POSTER 128 – SESSION 6.1

Convergent evolution of benzoxazinoid biosynthesis in three plant orders

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Plants synthesise a multitude of secondary metabolites. Variability seems to be essential for the function of these "specialised metabolites" in communication with the environment and as an arsenal for chemical defence. Establishment of secondary metabolic pathways is based on the common primary metabolism. Specific enzymes are then involved to generate the secondary metabolites. Benzoxazinoids are defence-related secondary metabolites found in three orders of the angiosperms: Poales, Ranunculales and Lamiales. These preformed defence compounds control broad spectra of microbial pathogens and herbivores. While the pathway is monophyletic in the grasses the branch point reaction to primary metabolism, the stabilisation of the toxic intermediate and bio-activation evolved independently by recruitment of homologous but not orthologous genes in the dicot and monocots. Phylogenetic analysis suggests that a pool of progenitor enzymes with substrate and reaction ambiguity was existing in the plant kingdom that allowed independent pathway evolution in the different orders. Enzyme promiscuity might be the basis for generation of secondary metabolite diversity in plants.

POSTER 129 – Session 6.1

Expression analyses of genes putatively involved in pyrrolizidine alkaloid biosynthesis in *Eupatorium cannabinum*

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Pyrrolizidine alkaloids (PAs) are compounds of secondary metabolism produced by the plants as a defense system. Homospermidine synthase (HSS) has been identified as first specific enzyme of PA biosynthesis. The gene encoding HSS originated by duplication from a gene encoding deoxyhypusine synthase (DHS), an enzyme from primary metabolism. HSS expression within Asteraceae is restricted to the roots and, in case of model plant *E. cannabinum*, correlated with the plant growth. We hypothesise that diamine oxidase (DAO family) are involved in the second step of PA biosynthesis. Putative *dao* candidate sequences have been identified from a subtractive cDNA library. Analyzes of expression levels suggest that some of the cDNA are coregulated with the cDNA encoding HSS. An *E. cannabinum* hairy roots system is presently used for RNAi approaches to test for the biological significance of these sequences for PA biosynthesis.

POSTER 130 – Session 6.1

Long term culture of somatic embryos in Nordmann fir leads to a decline in the yield of mature embryos - Consequences for practical application

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Nordmann fir (*Abies nordmanniana*) is of exceeding commercial value for the Christmas tree production in Europe. It is exclusively grown from seeds harvested from natural populations in the Caucasian mountains. This resource is limited and the resulting phenotype is not predictable. Hence, we induced numerous somatic embryo lines from selected trees to improve the quality and the cultivation protocol.

The biotechnical procedure for clonal mass propagation of Nordmann fir using somatic embryogenesis has been studied for more than 10 years and was improved with regard to synchronized maturation and cryopreservation. A remaining problem persists in long-term propagation of embryogenic cultures, which results in the reduction of numbers of normally developed mature embryos.

We adapted our current strategy characterizing lines from induction to acclimatization of plants by an as early as possible cryopreservation to produce stable quantities of somatic embryos over time. Optimized methods based on somatic embryogenesis are a realistic possibility to increase clonal variety and fulfil breeding requirements.

POSTER 131 – SESSION 6.1

Improvement of Nitrate-Use-Efficiency in Maize

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The use of nitrogen (N) fertilizers has dramatically increased in the last decades, especially for cereal crops. Because of high-energy costs and pollution maize plants (*Zea mays ssp. mays* L.) with minor fertilizer input are needed. There is a potential for improving of Nitrogen Use Efficiency (NUE) in the gene pool of maize. Two inbred lines investigated in this study differ in their adaptation to N supply. Previous analysis of the segregating population resulted in identification of two quantitative trait loci (QTL) influencing grain yield solely under low N conditions. In the current study we are trying to understand why one of the parental lines is more efficient than the other. Is the higher yield under low N conditions associated with a specific root system strategy? Root architecture of the parental lines and near-isogenic lines are analysed using three different plant growth systems: germination rolls, hydroponics and pots filled with sand. Because the evaluation of the quite low QTL effect in field trials is rather difficult, we are interested to find specific root phenotypes linked to the QTL and associated with NUE. That would substantially facilitate the QTL fine mapping and cloning. Our preliminary data suggest a possible association of the one QTL with altered parameters in lateral root growth and an association of the second QTL with specific characteristics of the crown roots growth.

POSTER 132 – SESSION 6.1

Transplantation of embryogenic cytoplasm into somatic cells of conifers by protoplast fusion

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The life cycle of economically interesting conifer species such as Douglas fir and Hybrid larch takes approx. 20 years - too long for efficient breeding programs based on classical methods. Somatic embryogenesis offers a promising alternative for large scale mass propagation of conifers. Unfortunately it is to date impossible to clone mature trees with known habitus efficiently, a fact that leads to high losses in time, money and elite breeding material. To overcome these limitations we are trying to transfer embryogenic competence to cells from adult tissues of elite-genotypes by protoplast fusion. As a base for this aim we succeeded in isolation, culture and regeneration of protoplasts derived from different sources such as embryogenic cells, buds and shoots. Using electric field-stimulated mass-fusion we obtained a high number of fusion-products, containing both parental cell types. Protoplasts were cultured and the regenerates that developed into somatic embryos were selected and identified by analyzing SSRs. Our results and improved methods present a feasible way to induce somatic embryogenesis on mature material, presenting a new chance to clone adult conifers.

POSTER 133 – SESSION 6.1

Direct somatic embryogenesis on leaf explants of *Dactylorhiza majalis spp. purpurella*

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Dactylorhiza majalis and *Dactylorhiza purpurella* are terrestric orchid species with rising demand in the horticulture industry. All prior developed orchid micropropagation methods, using shoot tips, leaf segments and other explants, were applied to the propagation of *Dactylorhiza* without success. In this study, we attempted in vitro leaf explant culture and report a successful method of protocorm-like body (PLB) induction. The effects of the growthregulators phytosulfokine-alpha (PSK-α) and spermidin, and the ethylen biosynthesis-inhibitors silver nitrate (AgNO3) and aminoethoxyvenylglycine (AVG) on protokorm-like body induction for different leaf explants of *Dactylorhiza majalis* ssp. *purpurella* were testet.The explants were taken from the base, the lamina and the tip of young leaves.PLB formation, number of surviving explants and region of PLB formation was recorded. Histological observation showedinduction of somatic embryos directly from epidermal and mesophyll cells of the leaf base without an intervening callus within 1 month when cultured on a GelriteTMbasal medium supplemented with 2,4-D, NAA, TDZ, Zeatin, PSK and AVG. Due to this study it is possible to propagate a large amout of plants for the horticulture industry.

POSTER 134 – SESSION 6.2

Analysis of genetic structure in a collection of synthetic derived and conventional bread wheat germplasm and association mapping for drought related traits

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Screening of wheat germplasm comprising synthetic derived (SBW), conventional (CBW) bread wheats and check cultivars (CCT) for drought tolerance was carried out through morpho-physiological and biochemical traits in hydroponics where stress was induced with PEG. The germplasm was evaluated for phenological traits in the field under well-watered and drought stress conditions imposed at pre anthesis stage for two successive growing seasons during 2010/11 and 2011/12 crop cycles. Overall, the performance of SBW was quite promising when compared to check cultivars. Some potential drought tolerant lines are recommended for further micro-yield trials. The germplasm was genotyped with 101 SSR markers for assessing its genetic diversity. Marker-trait association analysis was employed to identify SSR markers associated with traits related to drought. The stable estimate for the sub-populations (1-20) was carried out with Structure Software 2.2. TASSEL 2.0.1 was used to calculate Kinship Coefficients Matrix. In total, 61 marker-trait associations significant in both models were detected at $p \leq 0.01$. The intra-chromosomal position/location of several of these MTAs coincided with those previously reported whereas some were unique that had not been located to date. Opportunities for further wheat improvement are provided by these novel loci/MTAs based on a marker approach.

POSTER 135 – SESSION 6.2

Plant Seeds as a Productionsystem - comparative analysis of Plant made Pharmaceuticals (PMP) and Industrials (PMI) produced in different species

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Biodiversity in crop rotation might be increased by the additive production of high value compounds likePlant made Pharmaceuticals (PMP) and Industrials (PMI)in crops that are currently of more ecological the economic interest. As production platform plant seeds have several advantages such as high protein yield and stable storage of target proteins. The biopolymer Cyanophycin and a vaccine against rabbit haemorrhagic disease virus (RHDV) CTB::VP60 provide the model substances for our project. Arabidopis, Tobacco and pea serve as production plattforms. Two different seed specific promoters were used in comparison and fused either to the coding region for the Cyanophycin synthetase and of the CTB::VP60 gene. High amounts of the transgene encoded proteins could be achieved.

POSTER 136 – Session 6.2

Functional characterization of the Fra a gene family of strawberry

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The strawberry fruit proteins Fra a 1.01E, 1.02 and 1.03 are homologues of the major birch pollen allergen Bet v1. The strawberry proteins are known to have essential biological functions in pigment formation and ripening of strawberry fruits and seem to be responsible for allergic reactions to strawberry fruit. We expressed the different Fra a allergens in host organisms and evaluated the cross allergenic potential of the proteins in birch pollen allergic patients by a basophil activation test. Although Fra a 1.01E, Fra a 1.02 and Fra a 1.03 have sequence similarities of 70% with Bet v 1 Fra a 1.02 showed the highest allergenic potential of the three different Fra a isoforms and seems to be the major strawberry allergen. Small changes in amino acid sequence can change orthologs in their allergenic characteristics. White-fruited natural occurring strawberry mutants show a significant decreased level of Fra a 1 expression level, enzymes of the flavonoid biosynthesis pathway and metabolites. Some white-fruited strawberry varieties were screened using the anti-Fra a 1.02 antibody for hypoallergenic strawberry lines. Strawberry fruits with transient RNAi-mediated silencing of Fra a 1 isoforms in the red-fruited cultivar Elsanta show the same phenotype, expression pattern and a decreased concentration of metabolites like the white-fruited natural occurring strawberry varieties. The results of the study can serve as foundation for the development of hypoallergenic strawberry lines.

POSTER 137 – SESSION 6.2

Can a delay in senescence increase yield of potato plants with increased sink capacity?

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Because of the continuously increasing need for food and raw materials, it is of great importance to increase the yield of crops like potato plants. We addressed this issue when we created transgenic potato plants that overexpress the metabolite transporters GPT and NTT. Tuber yield and starch content were found to be increased in these plants (Zhang *et al.,* 2008). However, in field trials such an increase could not be observed. In addition, the plants showed an earlier senescence (Greiten, 2008) which led us to the current project. Gan and Amasino (1995) published an innovative strategy to delay leaf senescence, the SAG12::IPT system. To investigate the consequences of a possible delay of senescence for yield in GPT/NTT-overexpressing potato plants, SAG12::IPT is investigated to be additionally overexpressed in the background of GPT/NTT-overexpressing potato plants. To analyze whether the SAG12::IPT construct can delay senescence in potato plant, without negative additional effects, we transformed wild-type potato plants first. Furthermore, to study the promoter activity in potato plants we transformed wild type with a SAG12::GUS construct. Here, results of greenhouse-grown IPT- and GUS-potato plants are shown.

POSTER 138 – Session 6.2

Protein quality control - from protein recognition and degradation to conditional protein expression

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The ON/OFF status of *functional* proteins within a cell's proteome must be precisely controlled to ensure its proper life by checkpoint-like protein quality control (PQC) mechanisms. PQC also kicks in if proteins are "used up" after their action and thus need to be removed from the cell. In plants, PQC is important for breakdown of storage reserves in seeds, germination, leaf and shoot development, flower induction, cell division, and possibly plant-pathogen interaction. Functional plant proteins as one of the premier storage units for energy are hallmarks of plant development and their environmental stress tolerance, but also in the light of producing plant-made pharmaceuticals. We functionally analyze novel enzymatic components and substrates with special emphasis on the N-end rule (NERP). In plants, NERP is poorly understood. We have developed an *in vivo* transgenic protein stability reporter system. Our laboratory work is mainly focused on studies of enzymatic NERP components (E3 Ubiquitin ligases, arginyl-transferases, and amidases), their substrate proteins as well as on the use of protein expression "on demand" as biotechnological applications in plants. Therefore, we are establishing conditional stabilization/destabilization assays of diverse functional classes of proteins such as enzymes, transcription factors, storage and reserve proteins but also toxic and large proteins with difficult folds which might be used in molecular farming.

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POSTER 139 – SESSION 7.1

Providing a blueprint for PEP-CK type C4 photosynthesis in *Panicum maximum*

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Engineering a C3 crop to perform the C4 photosynthesis has been a major goal in the field over the last decades. However, a good understanding of molecular processes in the complex trait C4 is needed prior to genetic engineering. We performed RNAseq on two grasses, *Panicum maximum* (C4) and *Panicum clandestinum* (C3). In our study we present a complete blueprint for the C4 cycle in *P. maximum*, a PEP-CK+NAD-ME type plant. Focusing on the transport capabilities necessary to establish the needed flux, we propose three transport modules, which connect the different compartments. We used this model, confocal images, and gas exchange measurements to estimate possible limitations of establishing a C4 cycle in a C3 plant. Assuming that *P. clandestinum* is already well adapted, we found that per fixed molecule of CO2 *P. maximum* needs about 183-fold more intercellular transport processes, which is nearly double the increase in intracellular transport and soluble proteins. Species independent comparisons of the C4 syndrome have historically been difficult due to the use of different isoforms in different species. To circumvent this, we developed a strategy of complexity reduction. The reduction to gene functions as the main point of interest allows for a comparison independent of species variability. In a multiple species comparison we show this approach for Enzyme Classifiers and Protein families.

POSTER 140 – SESSION 7.1

The unusual chloroplast genome sequence of the Synchromophyceae-like alga *Chrysopodocystis socialis* (Ochrophyta)

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Chloroplast genomes play an important role in the understanding of organelle integration into the host cell, especially in the context of secondary or tertiary endosymbioses. Plastids of red algal origin can be found among haptophytes, cryptophytes, alveolates and heterokonts. Since previous attempts to sequence the plastome of the heterokont amoeboid alga *Synchroma grande* (Synchromophyceae, Ochrophyta) failed due to high amounts of DNA contaminants from co-cultivated bacteria, we attempted, for the first time, to enrich plastids of secondary red origin using fluorescence-activated cell sorting (FACS) prior to DNA extraction. To further increase DNA quantity whole genome amplification (WGA) was used. The first plastid genome of a Synchromophyceae-like alga, *Chrysopodocystis socialis*, is a circular molecule of ~126 kb with several unique features compared to the 15 heterokont algae plastomes sequenced so far. Two large inverted repeat regions of 16.6kb encase the smallest small single copy region (~ 2kb, 3 genes) reported. Also, a yet undescribed open reading frame was found, as well as a transporter usually only present in cryptophyte and rhodophyte plastomes. The presented chloroplast genome marks the foundation, upon which future sequencing attempts of the *Synchroma* species with chloroplast complexes can be based, e.g. using a long range PCR approach.

POSTER 141 – Session 7.1

R2R3-MYB genes in sugar beet

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High sucrose content of the taproot makes sugar beet (*Beta vulgaris* ssp. *vulgaris*) an important crop plant. Its genome was recently sequenced in a collaborative effort of MPI-MG in Berlin and the Centre for Biotechnology (CeBiTec) at Bielefeld University together with German plant breeders and funding from the BMBF. *B. vulgaris* is the first species sequenced from the order of Caryophyllales, which makes it interesting for comparative genomics. As a basis for automated gene prediction extensive manual annotation was required. To this end, we analysed the genome for its content of R2R3-MYB genes, which typically comprise more then 100 genes in plants and code for transcriptional regulators of metabolism, cell identity or stress responses. We manually annotated *R2R3-MYB* genes in *B. vulgaris* with the help of gene prediction programs, cDNA sequences and expert knowledge and proposed a classification and naming according to the one established for *Arabidopsis thaliana*. We analysed the chromosomal distribution of the genes, their structures and expression. Further steps include the cloning of full lengths cDNAs of putative homologs of genes with known function in *A. thaliana*. Functional characterisation of these cDNAs will be performed by complementation of corresponding *A. thaliana* mutants and transient transactivation assays of promoters from potential target genes in *A. thaliana* protoplasts.

POSTER 142 – SESSION 7.1

Sequencing of repetitive genomes: Is it possible to assemble complete genomes from next generation sequencing data?

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By the advent of next generation sequencing techniques it is feasible to sequence complete genomes without much working effort and at low costs when compared to Sanger sequencing. Several assemblers are available to generate draft genome sequences from billions of reads and the number of published genomes has been rising quickly. In an effort to clone a chilling tolerance QTL in maize, we sequenced several BACs by Illumina-, 454- and PacBio technology to achieve the complete continuous sequence of the QTL. Because of the highly repetitive structure of the maize genome, it was not possible to assemble complete BACs from Illumina or 454 data alone. The addition of long read information from PacBio sequencing revealed some mistakes in both, the Illumina- and 454- assemblies. Even having long reads, there were still problems in bridging repetitive regions because the quality of PacBio reads was too low to map most of the reads unequivocally to a certain repeat. Hence, we belief that the correct assembly of complete genomes requires not only large-insert clone libraries like BAC libraries but also high-quality long reads.

POSTER 143 – SESSION 7.2

Plant Metabolite Profiling at BASF

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Metabolite profiling plays a critical role at each stage of product development within BASF Plant Science. In addition to this "broad profiling" technology, which gives a comprehensive view of the metabolome, targeted methods are available to focus on particular metabolite classes based on project needs. Areas of application include the support of mode-of-action analyses, the use of metabolites as biomarkers, and the analysis of metabolites in the context of the development of compositional traits. As a first example, metabolite profiling was applied to investigate physiological base-tip gradients in corn leaves. The results clearly show that such gradients exist and that they are mirrored by transcriptomics and physiological data. As a second example, the technology was applied to investigate the mode-of-action of a new herbicide. Comparison of metabolic signatures showed that its mode-of-action differed from that of previously characterized herbicides. A deeper interpretation of the profiling data suggested a key metabolic step as the potential target site, which was subsequently confirmed by overexpression of a metabolic by-pass. In summary, the combination of our "cutting-edge" metabolite profiling platform with versatile data analysis and interpretation approaches creates unique opportunities to advance our projects across the product development pipeline at BASF Plant Science.

POSTER 144 – SESSION 7.2

Exploring the Arabidopsis sulfur metabolome

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Sulfur plays a crucial role in protein structure and function, redox regulation and plant biotic stress responses. Our understanding of sulfur metabolism, however, is limited to assigned pathways. The advent of high resolution mass spectrometry now makes it possible to assess the impact of natural and induced mutations on the metabolome. In this study, we present a high-resolution Fourier transform mass spectrometric approach in combination with stable isotope-labeling to describe the sulfur metabolome of *Arabidopsis thaliana*. Databases contain roughly 300 sulfur compounds assigned to *Arabidopsis*. We were able to recover only 37 of those 300 predicted compounds. By contrast, we identified approximately 140 sulfur metabolites that have not been assigned to the databases to date. We applied our method to characterize the γ-glutamyl-transferase mutant *ggt4-1*, which is involved in the vacuolar breakdown of GS-conjugates in detoxification reactions. Although xenobiotic substrates are well known, only few endogenous ones have been described. Among the specifically altered sulfur containing masses in the *ggt4-1* mutant, we identified one endogenous GS-conjugate and a number of further candidates for endogenous substrates. The high proportion of unassigned sulfur compounds we identified in this study emphasizes the need to reevaluate our understanding of the sulfur metabolome.

Session 7.3 – Systemic Approaches III: Proteomics and Phosphoproteomics

POSTER 145 – SESSION 7.3

Proteomic analysis of the Cyanophora paradoxa muroplast provides clues on early events in carbon allocation in plants

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Glaucophytes represent the first lineage of photosynthetic eukaryotes of primary endosymbiotic origin which diverged after plastid establishment. It is highly desirable to gain knowledge on the glaucophyte plastid composition in order to get insight into the evolutionary history of cyanobiont integration and plastid development. Here we provide the first proteomic analysis of the muroplast of *Cyanophora paradoxa*. Reconstruction of the ancient carbon fluxes from the cyanobiont to the host cytosol predicted that during endosymbiosis photosynthate was exported to the cytosol where it was polymerized from ADP-glucose into glycogen.The protein repertoire of the muroplast revealed novel paths for reduced carbon flow and export to the cytosol through a sugar phosphate transporter of chlamydial origin.Recently, Chlamydia-like pathogens turned out to be the second major source of foreign genes in Archaeplastida with a significant number of genes horizontally transferred. This hints at a significant role of these obligate intracellular pathogens during establishment of endosymbiosis, likely through facilitating the metabolic integration between the endosymbiont and the eukaryotic host. We here propose that an hexose phosphate transporter of chlamydial origin represented the first transporter responsible for exporting photosynthate out of the cyanobiont.

POSTER 146 – SESSION 7.3

A proteomic approach to unravel ricinoleic acid degradation

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Ricinoleic acid is an unusual fatty acid with a high value for industrial processes ranging from lubricants to the generation of Nylon 11. The main source is *Ricinus communis* (castor bean) that stores up to 80% of its seed reserves as ricinoleic acid. While its biosynthesis has been studied and used to induce ricinoleic acid generation in Arabidopsis, tobacco and yeast the degradation pathway is still elusive. The position of the hydroxylation prevents degradation via beta-oxidation past 2-hydroxyheptanoic acid. A landmark study performed by Hutton and Stumpf in 1971 presented its degradation to occur via a mix of alpha- and beta-oxidation in peroxisomes yielding propionate. Since the exact enzyme sets responsible for peroxisomal alpha-oxidation and propionate utilization in plants are not identified, we performed a proteomic analysis of organelles from *R. communis* endosperm. Organelle separation was accomplished by ultracentrifugation using a stepwise sucrose gradient. Fraction purity was analyzed by immunoblot detection of marker proteins and a suitable gradient chosen for the acquisition of proteomes. Based on the specific distribution of organelles within the obtained fractions, the subcellular localization of proteins was assigned with regard to peroxisomes, mitochondria and plastids. The acquired data was used to create a first draft of ricinoleic acid degradation in castor bean endosperm.

POSTER 147 – SESSION 7.5

The 2in1 system allows ratiometric BiFC and trafficking analysis

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Binary interaction techniques are vital tools that shape our understanding of protein complexes. An inherent flaw, however, with most current protein-protein interaction techniques is the variability in expression levels for fusion proteins when using several individual plasmids. We established a novel recombination-based cloning strategy - "2in1" - that enables co-expression of fusion proteins on a cell-by-cell basis from a single plasmid. 2in1 allowed the development of a ratiometric Bimolecular Fluorescence Complementation assay (rBiFC) [1] where both candidate genes are simultaneously cloned into a single vector backbone containing an internal fluorescent marker for expression control and ratiometric analysis. rBiFC significantly increases the credibility of protein-protein interaction results allowing ratiometric comparison between different protein pairs. In addition to its use in rBiFC, 2in1 can easily be introduced into other vector systems that rely on multiple gene expression and we have successfully implemented it in secretion analysis [2], FRET and Split-Ubiquitin assays.

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POSTER 148 – SESSION 7.5

Leading to accuracy: Function of Phragmoplast Orienting Kinesins

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In plants proper spatial control of cytokinesis is crucial to create the cellular network of tissues. The plane of division is predicted by the preprophase band (PPB), a cortical ring of cytoskeletal filaments. This spatial information has to be retained after the PPB disassembly, by establishing the cortical division site (CDS). During cytokinesis the phragmoplast (PP) facilitates cell plate assembly. It expands from the center of the cell outwards until the forming cell plate fuses with the cortical division site exactly. Accurate execution of division site establishment as well as PP guidance is essential for cell and plant morphology. Simultaneous mutation of *Phragmoplast Orienting Kinesin (POK) 1* and *2* leads to defective cytokinesis and consequently shows disorganized cell wall pattern in roots of *Arabidopsis thaliana*. Our phenotypic characterization revealed alterations in PP expansion. Furthermore localization studies indicate association with microtubules and support POKs involvement in division site establishment and function in PP guidance.

POSTER 149 – SESSION 7.6

"Next-generation" drought stress monitoring

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We present a novel measurement setup for monitoring leaf water status using non-destructive terahertz time-domain spectroscopy (THz-TDS). Previous studies showed the principal applicability of THz-TDS, with decreasing leaf water content directly correlating with increasing terahertz transmission. Using needles of silver fir (*Abies alba* Mill.) seedlings as test subjects, we could show that the transmission varies along the main axis of a single needle due to variation in tissue thickness. Therefore, any relocation of plants during the measuring period, which was necessary in the previous THz-TDS systems, should be avoided. Our new system overcomes such drawbacks while allowing for continuous monitoring of the water status of multiple individual plants, each of the plants at the same constant leaf position. Furthermore, we could show a highly significant correlation between gravimetric water content and respective terahertz transmission. Thus we were able to narrow down the permanent wilting point of the seedlings and ultimately establish groups of plants with defined levels of water stress that could not be detected visually. This opens up the possibility for a broad range of genetic and physiological experiments.

POSTER 150 – SESSION 7.6

Biochemical traits of plant cells measured by FTIR spectroscopy

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In plant ecology research the focus of plant traits shifting more and more towards chemical and biochemical cell composition. Since, the analysis of elemental cell quota or cellular content in proteins, major storage compounds like lipids and carbohydrates or structural components like lignin by means of biochemical methods is time consuming and needs relative high amounts of cell material, alternative measurements are recently under development. Fourier transformed infrared spectroscopy (FTIR) can overcome some of these limitations due to its high reliability, sensitivity, and the potential for high throughput analysis. We show that FTIR spectroscopy coupled to several statistic based data interpretation is a useful tool for determining biochemical traits of different plant material, ranging from small single cell phytoplankton up to higher plant samples. We will discuss the application of the method in different fields of plant ecology and biotechnology with respect to statistical data interpretation.

POSTER 151 – SESSION 7.6

Reduced nitrogen supply does not affect anthocyanin concentrations in red cabbage

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Red cabbage is increasingly interesting for dye production due to its high anthocyanin content (AC). Increasing the AC could probably be achieved by agricultural practices. This study aimed at elucidating whether decreased nitrogen (N) fertilization impacts the AC in red cabbage. Red cabbage `Lodero' (Bejo Zaden, Warmenhuizen, NL) was planted in the field in 2012. Half of the plots received 50% less calcium ammonium nitrate than controls ("Nred"). This resulted in a one-factorial experiment with four replications. Spectroscopic measurements (Multiplex, Force-A, France) were done on the peeled head after harvest. The same part was taken for chemical analyses. Total N was determined by the Kjeldahl procedure. Anthocyanins were extracted in 80% methanol and AC was determined photometrically (pH shift method). Spectroscopic measurements revealed no significant influences of the reduced N fertilization on N (NBI_G and NBI_R) and AC (FERARI, ANTH_RG and ANTH_RB) parameters. In contrast, chemical analyses showed that the concentration of N was significantly reduced in the "Nred" treatment ($p<0.001$) while there was no effect on the AC. The different results of spectroscopic measurements and chemical analyses are due to their weak correlation ($R2=0.01$-0.15 for AC, $R2=0.42$-0.46 for N). This shows that the N supply to red cabbage `Lodero' can be reduced by 50% without affecting the AC.

POSTER 152 – SESSION 7.6

Correlative Light and Electron Microscopy and Super-Resolution Array Tomography: Analysis of Secretory Vesicles in Arabidopsis Root Tip Cells

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Light microscopic analysis of plant cell secretory vesicle distribution and trafficking is greatly hampered by their small size (about 50-100 nm in diameter); also, there are very few reliable endogenous secretory markers available. We used ultrathin resin sections (50-70 nm) of cryofixed and freeze-substituted electron microscopy samples, ensuring close-to-native state preservation, for the labelling of a cell wall component as marker for secretory vesicles. We applied correlative light and electron microscopy in combination with array tomography with an axial resolution of about 70 nm for the three-dimensional analysis of the intracellular distribution.

POSTER 153 – SESSION 7.6

A plate reader based assay for vacuolar pH measurements in Arabidopsis

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Vacuolar pH measurements in Arabidopsis are usually time consuming and only allow measurement of a limited number of samples. We developed a plate reader based assay using a pH-sensitive fluorescent dye that enables in vivo quantification of vacuolar pH in Arabidopsis seedlings. The plate reader based assay allows processing of a large number of samples as well as recording kinetics of vacuolar pH after stimuli treatments. To gain more insight into mechanisms that contribute or regulate vacuolar acidification, we used the plate reader based assay to screen a library of more than 360 pre-screened bioactive compounds. Here we present first results demonstrating the usefulness of the plate reader based assay and candidate compounds that significantly alter vacuolar pH.

POSTER 154 – SESSION 8.1

Interactions of the glutamyl-tRNA reductase binding protein (GBP)

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Tetrapyrrole biosynthesis starts with the formation of 5-aminolevulinic acid (ALA) and leads to chlorophyll, heme, siroheme and phytochromobiline. Glutamyl-tRNA reductase (GluTR) is the first committed enzyme in ALA synthesis. Once glutamate is activated by ligation to tRNAGlu, GluTR reduces glutamyl-tRNA to glutamate-1-semialdehyde (GSA). Subsequently, GSA aminotransferase (GSAT) transaminates GSA to ALA. Expression and activity of GluTR are subjected to several transcriptional and posttranslational regulatory mechanisms. A GluTR-binding protein (GBP, previously called PROTON GRADIENT REGULATION 7) was identified as an interaction partner of GluTR (Czarnecki et al., 2011, Plant Cell). GBP is bound to thylakoid membranes and facilitates spatial separation into two pools of ALA synthesis for chlorophyll and heme biosynthesis, respectively. Little is known about the regulation and mode of action of GBP. We identified additional interaction partners of GBP in chloroplasts by means of approaches, such as bimolecular fluorescence complementation and yeast-two-hybrid experiments and continue elucidation of GBP function with different *Arabidopsis gbp* mutants and *GBP/GluTR*-overexpressing tobacco plants. Latter transformants are characterized by necrotic leaves indicating highly deregulated tetrapyrrole biosynthesis.

POSTER 155 – SESSION 8.1

GluTR2 substitutes GluTR1 activity, but is differently regulated at the posttranslational level

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Tetrapyrroles are prosthetic molecules that participate in fundamental biological processes, such as photosynthesis and respiration. Tetrapyrrole biosynthesis is tightly transcriptionally and posttranslationally controlled by environmental cues and endogenous factors, including metabolic feedback signals. Glutamyl-tRNA reductase (GluTR) is the first committed enzyme in the pathway and encoded by a small *HEMA* gene family in *Arabidopsis*. *HEMA1* is highly expressed in photosynthetic tissues, whereas *HEMA2* is constitutively expressed at a basal level in all tissues. The negative regulator FLU has been identified to interact with GluTR and is contained in a protein complex with four enzymes catalyzing final steps of chlorophyll synthesis (Kauss *et al*., 2012). Lack of FLU leads to enhanced protochlorophyllide contents in dark leaf cells. Interestingly, yeast two hybrid experiments proved that FLU interacts exclusively with GluTR1, but not with GluTR2 (Goslings *et al*., 2004). This regulatory distinction is conceivable and has intrigued researchers to assume that GluTR1 and GluTR2 are each associated with a certain portion of ALA-synthesis assigned either to chlorophyll or heme formation. Complementation experiments of the pale-green *hemA1* mutant, which is lethal under autotrophic conditions, are performed to examine compensatory consequences of GluTR2 expression.

POSTER 156 – SESSION 8.1

Dynamics of chloroplast proton motive force (*pmf*) partitioning in *Arabidopsis*

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Efficient photosynthesis involves the optimal use of absorbed photonic energy under limiting light conditions. Light-induced linear electron flow as well as proton transport across the thylakoid membrane generate the transmembrane proton gradient (ΔpH) and electric potential (ΔΨ), respectively. Together, the ΔpH and ΔΨ constitute the proton motive force (*pmf*) required for ATP synthesis. In higher plants, the rapidly inducible-reversible 'energy'-dependent qE component of non-photochemical quenching (NPQ) of excess light energy is largely controlled by the ΔpH. As such, the ΔpH and by extension the *pmf* play a central regulatory role in both photochemical and NPQ reactions. We are interested more generally in the relationship between *pmf* partitioning and the regulation of electron transport, NPQ and xanthophyll conversion at a wider range of light intensities (50-1200 µE). Simultaneous measurements of absorbance changes at 515 and 535 nm, as a probe of ΔpH and ΔΨ, respectively, and thus the *pmf* highlight a decrease in the total *pmf* and a parallel increase in the relative ΔpH component in wildtype *Arabidopsis* at all intensities, particularly at 50µE. Under these conditions, the ΔpH in isolation is not sufficient to drive NPQ at steady-state illumination, despite the requirement of a ΔpH early on during qE induction as well as long-term zeaxanthin formation. The general sequential dependence of NPQ and xanthophyll cycle regulation on the *pmf* and its constituent components will be further characterized.

POSTER 157 – SESSION 8.1

Localization and membrane interaction of the Zeaxanthin epoxidase of *A. thaliana*

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The xanthophyll zeaxanthin (Zx) has a central photoprotective function in the short- and long-term response of plants to high light stress. Zx is not only essential for the pH-regulated energy dissipation in the time range from seconds to minutes, but is also supposed to be involved in the down-regulation of photosystem II at longer time scales (up to several hours). In addition to that, Zx is known to contribute to photoprotection independent of its role in energy dissipation. The Zx epoxidase (ZEP) activity and hence the reconversion of Zx to violaxanthin was found to be gradually down-regulated with increasing light stress. These characteristics imply that Zx may act as a molecular memory of light stress, although the molecular basis is not understood. Zx serves not only as photoprotective xanthophyll but also as precursor of the stress hormone abscisic acid (ABA). To date, the exact localization within the chloroplast and the molecular basis of the light-dependent regulation of the ZEP are largely unknown. Using specific antibodies raised against the ZEP of *Arabidopsis thaliana*, we have analyzed the localization and distribution of the ZEP protein. Furthermore, we studied its interaction with the thylakoid membrane. Our data provide new insights into the localization and light regulation of the ZEP in relation to its function in energy dissipation and ABA biosynthesis.

POSTER 158 – SESSION 8.1

A photosynthetic *Arabidopsis thaliana* mutant is partially rescued by a transcription factor (TF) from the C4 plant *Cleome gynandra*

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An *A. thaliana* mutant lacking a photosynthetic TF is disturbed in the development of chloroplasts and photosystems. In this work, we ask if the TF homologues from a C3 and C4 plant control a different set of genes. To compare the function of the homologues, we rescued an *A. thaliana* knockout mutant with the C4 homologue and analyzed the chlorophyll level. Furthermore, we treated the plants with high light (1500 µE) and measured the Fv/Fm value to determine differences in photoprotection. We found that the chlorophyll content was restored to wildtype level in rescued mutants. However, photoprotection was disturbed in the mutants and did not recover in the rescued lines. The analyzed mutant is not recovered completely by heterologous expression of its C4 homologue. From that we conclude that it addresses a partially changed set of genes, which will be analyzed in future experiments. The changed specificity of the TF is likely to contribute to the C4 syndrome, where photosynthetic requirements are different from that of C3 plants.

POSTER 159 – SESSION 8.1

Acclimation of Arabidopsis thaliana to different growth light conditions: impact on energy dissipation and thylakoid membrane organization

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Energy uptake in plants takes place at the level of the light-harvesting complexes (LHCs) of photosystem I (PSI) and II (PSII) in chloroplasts. More energy is often absorbed than can be used for photosynthesis, leading to the formation of damaging reactive oxygen species (ROS). Non-photochemical quenching (NPQ) effectively reduces photooxidative damage by dissipating excess energy as heat. This requires (i) acidification of the thylakoid lumen, (ii) PsbS-mediated LHC conformational changes, and (iii) the binding of Zeaxanthin to specific sites in the antennae. Plants grown under different light conditions possess a specific assembly of antennae proteins and size that allow for reorganization of the antenna super-complexes according to incoming light energy. The overall number of LHCs formed is inversely related to the given growth light intensity. We are interested in the structural changes of PSII antenna in response to growth light intensities, ranging from low to high as well as natural light conditions in particular, which are acclimated to fluctuating light compared to those grown under controlled conditions. Analyses of fluorescence quenching indicate a higher quenching capacity in plants grown under high compared to low light. Under natural light conditions, plants appear to possess intermediate quenching capacity and overall faster induction kinetics. Additional pigment stoichiometry data will offer deeper insight into the development of photosynthetic capacity.

POSTER 160 – SESSION 8.1

The mysterious rescue of *adg1-1/tpt-2* - an *Arabidopsis thaliana* double mutant impaired in acclimation to high light - by exogenously supplied sugars

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An *Arabidopsis thaliana* double mutant with a knock-out in the triose-phosphate/phosphate translocator (TPT; *tpt-2*) and a defect in starch biosynthesis in leaves (AGPase; *adg1-1*) exhibits growth retardation and a "high chlorophyll fluorescence" (HCF) phenotype under high light conditions. Both phenotypes could be rescued when grown on sucrose (Suc) or glucose (Glc). Triple mutants additionally defective in the Glc-sensing hexokinase1 (HXK1; *gin2-1*) or the glucose 6-phosphate/phosphate translocator 2 (GPT2; *gpt2-1*) were used to investigate the involvement of both genes in the sugar-dependent rescue. Wild-type plants, single, double and triple mutants were grown on agar in the presence or absence of each Suc, Glc, or fructose (Fru). The growth phenotype of all double and triple mutants could be rescued only by Glc and Suc. All three sugars were able of rescuing the HCF and photosynthesis phenotype, independent of HXK1. It appears likely that the direct up-take of soluble sugars into chloroplasts and their use as alternative source for anabolic processes within the stroma rescues the photosynthesis phenotype of *adg1-1/tpt-2*. The impact of sugar turnover and probably signalling inside the chloroplasts for the concept of retrograde signalling is discussed.

POSTER 161 – SESSION 8.1

Heterogeneity of NPQ indicates mechanistic differences in the process of enhanced heat dissipation in five green algal species

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Non-photochemical quenching (NPQ) is the fast response to high light stress and in that matter important for the rapid dissipation of excess excitation energy, a common requirement for photosynthetically active organisms. At present it is unclear whether NPQ is related to the phylogeny of plant species or to the selection pressure of the environment. Therefore NPQ was studied in five different green algae, isolated from different habitats, by means of the analysis of Chl a fluorescence, of the pigment composition and by the determination of the proton gradient across the thylakoid membrane. The results show, that the structural basis of NPQ is heterogeneous in the five species investigated. While the biofilm-forming green algae *Bracteacoccus minor*, *Chlorella saccharophila* and *Chlorella vulgaris* exhibit a zeaxanthin-dependent NPQ, in the planktonic green alga *Pedinomonas minor* and in the biofilm-forming *Tetracystis aeria* the zeaxanthin-dependent NPQ is completely absent. *T. aeria* still exhibits a moderate NPQ due to a strong proton gradient, whereas the inefficient NPQ of *P. minor* is characterized by a low proton gradient during high light illumination. The analysis of the data shows that NPQ is rather related to the selection pressure of the environment than to the phylogeny of the algae.

POSTER 162 – SESSION 8.1

The *hcf107* mutation of *Arabidopsis thaliana* can be complemented by a compartment-alien transformation with the plastome-encoded *psbH* gene

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High chlorophyll fluorescence-mutants of *Arabidopsis thaliana* are used to identify factors, which are important for the biogenesis of PSII. In one of these mutants, *hcf107,* PSII subunits are strongly reduced, especially PsbH and CP47 (PsbB). Consequently, the *hcf107* mutant is not able to grow photoautotrophically. Both of these PSII subunits are encoded by plastid genes and are part of the polycistronic *psbB*-operon. The lack of PsbH correlates with the absence of those RNAs in which *psbH* is the leading cistron, indicating that only these RNAs are fundamental for PsbH translation. In contrast, the amount of *psbB* RNA is not affected. This raises the question, if the reduction of CP47 protein is a secondary affect due to the absent PsbH. To test this hypothesis we attempted to provide PsbH protein to *hcf107* chloroplasts by equipping the gene with the plastidial targeting sequence of the PsbS protein and the 35S promoter. This chimeric gene was inserted into the genome of the *hcf107* mutant. We found that the nuclear-localised chimeric *psbH* gene was able to complement the mutant defect resulting in plants that grew photoautotrophically. Further experiments showed that cytosolically synthesized PsbH protein was assembled into PSII complexes. This is the first time in which a defect in a plastome-encoded hydrophobic membrane protein could be rescued by a compartment-alien transformation with that gene.

POSTER 163 – SESSION 8.1

A non-intrusive indicator for the violaxanthin cycle pool size in leaves

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Carotenoids are integral parts of the pigment protein complexes in green plants. In the light harvesting complexes (LHCs) xanthophylls have roles in light harvesting and dissipation of chlorophyll triplet states. The pigments of the violaxanthin cycle mediate thermal dissipation of excessive excitation energy. Xanthophylls bound to the violaxanthin binding site (V1-site) in LHCII have been shown not to participate in light harvesting. The total amount of the xanthophylls belonging to the xanthophyll cycle, i.e. violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), varies with growth irradiance of the plants by a factor of up to five. Since carotenoids absorb blue, but not red light, the growth irradiance dependent variations in xanthophyll to chlorophyll stoichiometry should result in a changed shape of fluorescence excitation spectra with decreased relative excitation efficiency in the blue wavelength region. We tested the hypothesis that the fluorescence excitation efficiency ratio of blue to red light (F(B)/F(R) declines with increasing violaxanthin cycle pool size (VAZ) by growing *Arabidopsis thaliana* tt3 mutants lacking anthocyanin formation at high irradiance and low temperature. We found an excellent linear correlation between F(B)/F(R) and VAZ. F(B)/F(R) may be used as a rapid nonintrusive indicator of light acclimation of the photosynthetic apparatus.

POSTER 164 – SESSION 8.1

Two ferredoxin isoproteins are essential for effective photosynthetic electron transport

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Ferredoxin (Fd) is the first soluble electron acceptor in the photosynthetic electron transport (PET) chain. Fd is capable of distributing electrons to many stromal acceptors, or returning them to the PET chain in a cyclic electron flow (CEF). CEF allows the generation of a ΔpH, without the release of electrons into the chloroplast stroma. All higher plants for which we have significant sequence information contain at least two photosynthetic Fd isoproteins, but it is not yet clear whether they are functionally differentiated. In Arabidopsis, the major isoform (Fd2) contributes to more than 90% of the total leaf Fd content, while the portion of the minor isoprotein (Fd1) is less than 10%. Based on preliminary analysis of RNAi lines, specific functions for the two different Fds were hypothesized: That Fd2 is predominantly involved in reduction of NADP+, while Fd1 has a specific regulatory function in CEF. In this work, we used knock-out mutants of Fd1 to thoroughly test this hypothesis. Our data show that Fd1 is essential for effective PET and that knock-out results in severe phenotypic and photosynthetic disturbance. In addition, our results indicate a specific function of Fd1 in regulatory CEF around PSI, suggesting that the presence of two different photosynthetic Fds in higher plants may provide a mechanism for regulating the switch between linear PET and CEF.

POSTER 165 – SESSION 8.1

The impact of an ER located protein on chloroplast biogenesis in *Arabidopsis thaliana*

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It is known that plastid development rely on the interaction with the endoplasmic reticulum (ER). Close contact sites between the envelope and the ER membrane system are proposed to constitute a major lipid trafficking route from the ER to the plastid. In addition, a secretory pathway was identified, which is necessary for transport of glycosylated proteins from the ER into the chloroplasts. How these interactions between both cell organelles are established is not clear so far. In addition the relevance of transported components is only partly known. In a forward genetic screen for chloroplast defective phenotypes we isolated the high chlorophyll fluorescence mutant *hcf222*. Spectroscopic measurements revealed a defect in the intersystem photosynthetic electron transport. Detection of diagnostic protein subunits of the photosynthetic membrane complexes reflected reduced accumulation of proteins from the cytochrome *b6f* complex. We mapped the mutated gene and confirmed its identity by complementation analysis. *HCF222* encodes a protein, which is conserved in higher plants and *Physcomitrella* but has no orthologs in algae and cyanobacteria. The transient expression of an HCF222-GFP fusion in *Nicotiana benthamiana* revealed the localization of this protein in the ER. On the poster the function of HCF222 is discussed in correlation to the observed phenotype of the mutant.

POSTER 166 – SESSION 8.1

Identification of Membrane-Bound RNAs in Chloroplasts

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Chloroplast gene expression is characterized by a multitude of RNA processing events that have been scrutinized over the last three decades by various techniques. We know details about the many individual processing events occurring on chloroplast RNAs and have begun to understand the machinery behind transcription, RNA processing and translation. By contrast, we know close to nothing about the spatio-temporal dynamics of chloroplast gene expression. Early studies already suggested that chloroplast RNAs can be immobilized on membranes, but details on the nature of the bound RNAs was lacking. In this study, we have looked into sub-organellar RNA localization on a transcriptome-wide scale. We found that many mRNAs coding for proteins of the photosynthetic machinery are associated with thylakoid membranes. Quantitative analyses suggest that spliced mRNAs are associated with membranes, while unspliced mRNAs are free in the stroma. This indicates that a quality-control step for chloroplast mRNAs exists that prevents unprocessed mRNAs from shifting to (presumably translated) complexes on thylakoid membranes.

POSTER 167 – SESSION 8.1

The Roles of Low-Molecular-Weight Subunits in Biogenesis, Structure and Function of Thylakoid Membrane Complexes

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Generation, maintenance and repair of the thylakoid membrane system are highly sophisticated dynamic processes. To elucidate the biogenesis as well as the structure/function relationship of this system we inactivated 19 plastid genes encoding thylakoid membrane proteins using a transplastomic approach in tobacco. We paid special attention to low-molecular-weight subunits (LMWs) of both photosystems and the cytochrome *b6f* complex since their roles in photosynthesis and assembly processes remained largely unknown. Photosynthetic performances, dynamic posttranslational processes, like the reversible dissociation/association of chlorophyll complexes from the photosystems (state transition) and the redox-dependent regulation of phosphorylation as well as the time-resolved assembly of complexes and the turn-over of individual subunits were investigated. Interestingly, many of these mutants are still viable but distinct lesions could be detected in individual mutants. We identified those LMWs important for the dimerisation of photosystem II and the cytochrome *b6f* complex. LMWs were also found to be crucial for the stability and diverse functions of the complexes, like light trapping, oxidation of plastoquinone, charge recombination, forward and backward electron flow. Novel data about the localisation, the topology and the function of the enigmatic PsbN protein in accumulation of PSII will be presented.

POSTER 168 – SESSION 8.2

Approaches to increase plant biomass through optimization of net photosynthesis

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The weather extremes expected due to climate change and global warming lead to heavy stress for crop plants and in consequence to yield reduction. Photosynthesis is one of the first processes which is influenced by such abiotic stresses. In order to guarantee a consistent supply of energy, which is independent of abiotic stress factors, tobacco plants were created, which have a modified photosystem II and their stress reaction is analysed. In addition, in order to use this energy in biomass production, an enzyme that should improve the performance of the Calvin cycle was expressed in tobacco plants. Due to these modifications we hope to minimize the stress induced yield reduction.

POSTER 169 – SESSION 8.2

Photorespiration in red algae: Functional analysis of the glycolate oxidase in *Cyanidioschyzon merolae*

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Photorespiration is essential for organisms performing photosynthesis. The photorespiratory metabolism is of cyanobacterial origin and has evolved to a highly specialized pathway in higher plants. The study of photorespiration in eukaryotic algae, such as glaucophytes, red and green algae will help to understand the evolution of this pathway. However, details on the photorespiratory pathway in red algae are still scarce. In order to study the function and importance of photorespiration in red algae we use the model strain *Cyanidioschyzon merolae*. Its genome is fully sequenced and tools for targeted gene mutation and protein localization are available. As our first target we chose the glycolate oxidase (GOX). The conversion of glycolate to glyoxylate is a central step, which is performed either by glycolate dehydrogenase (GlcD) in cyanobacteria and some green algae or by GOX in higher plants. It is assumed that GOX mainly exists in organisms with a high photorespiratory flux over different organelles. In contrast GlcD exists in organisms that lack peroxisomes and employ a carbon concentrating mechanism. Using YFP-GOX fusion proteins we can demonstrate, that GOX is targeted to peroxisomes in *C. merolae*. Currently, a *GOX* knock-out mutant is generated. Its physiological characterization under different CO2 concentrations will shed light on the importance of photorespiration in red algae.

POSTER 170 – Session 8.2

Evolution of C4 Photosynthesis in the Genus *Flaveria* - Establishment of the photorespiratory CO2 pump

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In C4 plants RubisCO is exclusively located in the bundle sheath (BS) cells. C4 photosynthesis is essentially a CO2 concentrating mechanism that reduces the oxygenase activity of RubisCO through an initial fixation of CO2 by a second carboxylase without an oxygenase function. A further process restricted to the BS cells in C4 plants is photorespiration, which is needed to recycle the product of the oxygenase reaction of RubisCO thereby releasing CO2. One of the key enzymes of photorespiration is glycine decarboxylase (GDC). To analyze the evolutionary adaptations of the BS restriction of GDC the expressional changes of one of the subunits, GLDP, was investigated in the transition from C3 to C4 in the genus *Flaveria*, including C3-C4 intermediates. Three groups of *GLDP* genes were found, one of these groups contains genes that are expressed BS specifically in the C3 species already. The 5' flanking sequence of the BS specific *GLDPA* gene of the C4 species *F. trinervia* contains a tandem promoter, which is conserved in all genes of this group. In contrast to the C4 species in the C3 species the distal promoter is silent and thus became activated in the transition from C3 to C4. At the same time a second *GLDP* gene that is expressed ubiquitously in the C3 species became gradually inactivated in the course from C3, through intermediates and is fully inactive by pseudogenization in the C4 species.

POSTER 171 – SESSION 8.2

Separation and systems analysis of bundle sheath and mesophyll cells along maize leaf gradient.

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C4 photosynthetic plants shuttle carbon from an exterior to an interior tissue, concentrating it around Rubisco. The basic cycle is well understood and several recent developmental and mature tissue-specific systems analyses have increased our understanding of C4 photosynthesis. Yet, we still lack tissue-specific resolution of the gene expression and metabolic environment in immature maize tissue. This study attempts to understand how bundle sheath and mesophyll tissues develop, how the C4 cycle becomes integrated, and what important regulators might be active in a developing maize leaf. To this end, a maize leaf gradient was harvested, and the two cell types were enriched via filtering ground material over serially-smaller meshes cooled with liquid nitrogen. The enriched material was used for measurement of enzyme activity, metabolic analysis, and RNA sequencing. While analysis is still in progress, we see a good correlation with previous studies and knowledge. Further, this systems analysis has already revealed different expression and activity modules along the leaf gradient. Some transcripts show fairly constant enrichment as the leaf develops, while others show enrichment varying along the leaf gradient. Notable among the patterns are two distinct clusters with genes—including many transcription factors—enriched in the base segment for BS and M expression, respectively.

POSTER 172 – Session 8.3

Genetic analysis of de novo and salvage pathways of nucleotide sugars for plant cell walls

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Cell wall polymers are synthesized by glycosyltransferases using nucleotide sugars as glycosyl donors. We are studying two de novo pathways for the synthesis of UDP-glucuronic acid, involving UDP-glucose dehydrogenase (UGD) [1] and myo-inositol oxygenase (MIOX) [2] as key enzymes. This nucleotide sugar is the common precursor for apiose, arabinose, galacturonic acid and xylose in cell wall polymers. Analysis of knockout mutants indicates a dominant role of UGDs for precursor biosynthesis of cell wall polymers. A reduction in MIOX activity is compensated by the UGD pathway and therefore causes no changes in the cell wall of miox knockdown mutants. However miox mutants are more resistant to nematode infections. We have recently also focused on salvage pathways for UDP-sugars. Some of the enzymes are essential for pollen development. The analysis of mutants surprisingly reveals a much more important role of nucleotide sugar recycling as previously thought [3].

[1] R. Reboul et al. (2011) J. Biol. Chem., 286, 39982-92.

[2] S. Endres and R. Tenhaken (2011) Planta, 234, 157-169.

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POSTER 173 – SESSION 8.3

Arabidopsis G6PD1 and PGL3 isoforms are targeted to chloroplasts and/or peroxisomes by interaction with Trx *m2* in the cytosol

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During analysis of the oxidative pentose-phosphate pathway (OPPP) in Arabidopsis we found that plastidic glucose 6-phosphate dehydrogenase G6PD1 and 6-phosphogluconolactonase PGL3 localize to both plastids and peroxisomes and that the targeting switch is regulated at a post-translational level. Yeast 2-hybrid analyses indicated that thioredoxin *m2* (Trx*m2*) is also involved in regulating targeting of PGL3, as previously shown for peroxisome targeting of G6PD1 (via catalytically inactive isoform G6PD4). In planta bimolecular-fluorescence complementation confirmed that Trx*m2* interacts with PGL3 in chloroplasts and in the cytosol, but not in peroxisomes. Co-expression analyses further supported that chloroplast import of PGL3 involves Trx*m2*, and Cys-to-Ser changes in Trx*m2* showed that PGL3 binding occurs independent of redox-state. In leaves, dual-targeting of PGL3 to plastids and peroxisomes was detected in mesophyll cells but not in the epidermal cell layer, where Trx*m2* expression was undetectable. Thioredoxins were lately shown to exhibit chaperone function, acting as holdases (in multimeric form) but upon redox-activation as foldases (in monomeric form). Thus, interaction with Trx*m2* in the cytosol can explain coordinated subcellular targeting of G6PD1 and PGL3 by either preventing or promoting folding of PGL3 precursors, depending on cytosolic redox-state.

POSTER 174 – Session 8.3

A systems biology analysis of early-stage sugar beet storage root development

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In sugar beet (*Beta vulgaris* L.), sucrose is not only the major transport form of assimilates, but it also accumulates at high concentrations in storage roots. Since sugar beet studies so far concentrated on source-sink relations in mature plants with a fully developed storage root, the metabolic changes occurring during the initial phase of sugar beet storage root development have not been systematically addressed. In the presented study, a systems biology analysis of early-stage sugar beet storage root development was conducted. The activities for different key enzymes of carbohydrate metabolism were analyzed in developing storage roots over the first 80 days after sowing, complemented with an *in situ* localisation of selected enzyme activities, expression analyses for the respective transcripts, anatomical investigations, and soluble sugar, hexose-phosphate and phytohormone profiles. Based on the accumulation dynamics of biomass and sucrose, as well as on anatomical parameters, the early phase of storage root development can be subdivided into two stages (prestorage stage; secondary growth and sucrose accumulation stage), each of which is characterised by distinct metabolic, transcriptional and phytohormonal signatures. The onset of secondary growth and sucrose storage is preceded by a phase of metabolic transition.

POSTER 175 – SESSION 8.3

Characterization of cytosolic pyruvate kinase family in Arabidopsis thaliana

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Cytosolic pyruvate kinases (cPKs) catalyze the the final step of glycolysis, the conversion of phosphoenolpyruvate (PEP) into pyruvate, thereby releasing ATP. Despite their pivotal role in plant primary metabolism to date cPKs in *Arabidopsis* have not been studied so far. The *Arabidopsis thaliana* genome encodes 14 PK genes whereas only 3 cytosolic isoforms named as PK1, PK2 and PK3 are expressed up to a reasonable level. Public expression data show a dissimilar pattern for these isogenes. Whereas PK2 and PK3 exhibit redundancy concerning tissue specificity and their response to diverse stresses, PK1 seems to have a unique function in this group. Investigation of respective T-DNA insertion-lines for metabolites related to plant respiration and localization of PK-YFP-fusion constructs will clarify the function of the different PK isoenzymes within the cytosol. Notably only PK2 und PK3 are expressed in response to pathogen attack. Elucidating the role of PK activity will give one of the first links between primary metabolism and plant defense response.

POSTER 176 – SESSION 8.3

SRT2, the *Arabidopsis* class II sirtuin, is a lysine deacetylase and interacts with mitochondrial energy metabolism

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The post-translational regulation of proteins by lysine acetylation has recently emerged to occur not only on histones but also on organellar proteins in plants and animals. In particular the catalytic activities of metabolic enzymes have been shown to be regulated by lysine acetylation. The Arabidopsis genome encodes two predicted sirtuin-type lysine deacetylases of which only SRT2 contains a predicted presequence for mitochondrial targeting. Here we have investigated the function of SRT2 in *Arabidopsis thaliana*. We were able to demonstrate that SRT2 functions as lysine deacetylase. We show that SRT2 resides in mitochondria and interacts with a small number of protein complexes mainly involved in energy metabolism and metabolite transport. The *srt2* plants display no growth but rather a metabolic phenotype. Our results indicate that SRT2 is important in fine-tuning mitochondrial energy metabolism.

POSTER 177 – SESSION 8.3

Identification of lysine acetyltransferases in *Arabidopsis* thaliana

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The εN-acetylation of lysine (K) side chains is a reversible and highly regulated post-translational modification of both prokaryotic and eukaryotic proteins. It has a strong impact on the biological function of proteins as the transfer of the acetyl group to lysine eliminates the positive charge which is known to be important in many catalytic centres of enzymes, as well as for protein-protein, and protein-DNA interaction. Lysine acetylation as described here is functionally different from the αN-acetylation which occurs during translation on more than 80% of all proteins. Specific protein acetyltransferases and deacetylases catalyse the reversible modification of the εN-group of lysine by transfer of an acetyl moiety from acetyl-CoA. In addition to histones and transcriptional regulators, several proteins involved in photosynthesis such as Calvin cycle enzymes but also proteins of the light reactions were found to be lysine-acetylated in Arabidopsis leaves (Finkemeier et al., 2011; Wu et al., 2011). This study aims to identify lysine acetyltransferases in Arabidopsis by using subcellular localisation analysis and co-immunoprecipitation approaches.

POSTER 178 – Session 8.3

Redox-regulation of the multifunctional cytosolic enzyme GAPDH involved in redox-signaling in Arabidopsis

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Apart from glycolysis for energy production, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) participates in many other functions in mammals by its diverse activities accompanied by changes in its subcellular localization. The various subcellular localizations are usually associated with post-translational redox-modification of GAPDH, and these changes subsequently lead to the transient microcompartmentation and transcriptional changes in the nucleus which trigger cell death. In plants, upon oxidative stress GAPDH was localized and accumulated in the nucleus and its high redox-sensitivity may play a role in oxidative stress signaling or protection. However, the function in the nucleus and the translocation mechanism of GAPDH is unknown. In this study, the nuclear localization of two cytosolic isoforms of phosphorylating GAPDH from *A. thaliana* (GapC1, GapC2) in oxidatively stressed protoplasts was directly visualized by BiFC. From differences found for the two isoforms, we assume that GapC1 and GapC2 are individually involved in the redox-signal transduction. Function of GapC isoforms under various redox-states, its regulation by redox-modification and microcompartmentation of GapC currently being analyzed. In addition, structure predictions for *A. thaliana* GapCs were used to get more insight into the potential difference of the two isoforms.

POSTER 179 – SESSION 8.3

The phosphoserine pathway is essential for plant development and metabolism

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Serine represents an essential constituent of proteins and plays an important role as precursor for the biosynthesis of other metabolites. Plants possess two independent serine biosynthetic pathways, the photorespiratory and the glycolytic phosphoserine pathway. In contrast to the photorespiratory pathway only little information are available on the function of the phosphoserine pathway (PS) in plants. In our study, we present a detailed characterization of the phosphoglycerate dehydrogenase (PGDH) as a component of the PS-pathway in *Arabidopsis thaliana*. The analysis of *pgdh1* mutants revealed an embryo lethal phenotype and PGDH1 miRNA lines were retarded in growth. Studies of metabolite levels in PGDH1 miRNA lines grown under ambient and elevated CO2conditions indicate a direct link between phosphoserine biosynthesis and ammonium assimilation. Furthermore, the expression of several genes of the PS-pathway is regulated by MYB51, an activator of tryptophan and indolic glucosinolate biosynthesis. Detailed studies revealed reduced content of tryptophan-derived glucosinolates and activity of the auxin-sensitive *DR5* promoter in *PGDH1*miRNA plants. Taken together, our results provide evidence for an substantial function of the phosphoserine biosynthesis in plant development and metabolism.

POSTER 180 – SESSION 8.4

HETEROMERIC AND HOMOMERIC GERANYL DIPHOSPHATE SYNTHASESFROM CATHARANTHUS ROSEUS AND THEIR ROLE IN MONOTERPENE INDOLEALKALOID BIOSYNTHESIS

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Geranyl diphosphate (GPP), the entry point to the formation of terpene moiety, is a product of the condensation ofisopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by GPP synthase (GPPS). Here, we report three genes encoding proteins with sequence similarity to large subunit (CrGPPS.LSU) and small subunit (CrGPPS. SSU) of heteromeric GPPSs, and a homomeric GPPSs. CrGPPS.LSU is a bifunctional enzyme producing both GPP and geranyl geranyl diphosphate (GGPP), CrGPPS.SSU is inactive, whereas CrGPPS is a homomeric enzymeforming GPP. Co-expression of both subunits in Escherichia coli resulted in heteromeric enzyme with enhanced activity producing only GPP. While CrGPPS.LSU and CrGPPS showed higher expression in older and younger leaves, respectively, CrGPPS. SSU showed an increasing trend and decreased gradually. Methyl jasmonate (MeJA) treatment of leaves significantly induced the expression of only CrGPPS.SSU. GFP localization indicated that CrGPPS. SSU is plastidial whereas CrGPPS is mitochondrial. Transient overexpression of AmGPPS.SSU in C. roseus leaves resulted in increased vindoline, immediate monomeric precursor of vinblastine and vincristine. Although C. roseus has both heteromeric and homomeric GPPS enzymes, our results implicate the involvement of only heteromeric GPPS with CrGPPS.SSU regulating the GPP flux for MIA biosynthesis.

POSTER 181 – SESSION 8.4

Deciphering the primary metabolic pathway of purine nucleotide catabolism

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In natural ecosystems, plants frequently grow under nitrogen limitation. Not only effective uptake and assimilation mechanisms but also biochemical pathways for internal reallocation of nitrogen are required for efficient utilization of this scarce resource. Using bioinformatic approaches coupled with biochemical studies and metabolic analyses of mutants, we have identified several enzymes which are involved in the remobilization of nutrients from purine nucleotides. This presentation focusses on guanosine deaminase (GSDA), an enzyme unique to plants, which catalyzes the deamination of guanosine to xanthosine. Surprisingly, metabolite and phenotypic analyses of several Arapidopsis single and double mutants revealed that purine nucleotides dedicated for degradation are channeled almost exclusively through GSDA. This demonstrates - contrary to the current view - that a non-branched, linear pathway of purine nucleotide catabolism exists in vivo which begins at GMP. The described pathway is particular to plants and dissimilar to purine nucleotide degradation in animals or microbes.

POSTER 182 – Session 8.4

Biochemistry of Glucosinolate Breakdown: Towards a better understanding of Specifier protein structure and Function

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Specifier proteins are part of the glucosinolate-myrosinase defense system of the Brassicaceae. Upon tissue damage, myrosinases hydrolyse glucosinolates to toxic isothiocyanates. In the presence of specifier proteins, breakdown is redirected to alternative products such as nitriles, epithionitriles or thiocyanates. Question: How do specifier proteins work biochemically? Methods: A purification protocol was established for recombinant Arabidopsis thaliana nitrile-specifier protein (AtNSP3-His6) and thiocyanate-forming protein from Thlaspi arvense (TaTFP, GST-tag removed) to obtain sufficiently pure protein for structure elucidation and interaction studies with purified Sinapis alba myrosinase using Mts-Atf-Biotin as a photoactivatable trifunctional crosslinker. Results: TaTFP has been purified to homogeneity at 2 mg/ml and is being used for crystallography. Incubation of Mts-Atf-Biotin-labeled myrosinase with AtNSP3-His6 or TaTFP at 366 nm resulted in crosslinking and label transfer after DTT treatment. Deletion of the lectin-domain of AtNSP3 did not interfere with label transfer. Conclusion: Myrosinase interacts with specifier proteins at a distance of <11.1Å, and this interaction is independent of lectin-domains. Experimental structure elucidation has become a realistic possibility.

POSTER 183 – SESSION 8.4

How do realistic ozone concentrations affect the biosynthesis of rosmarinic acid in *Melissa officinalis*?

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Lemon balm (*Melissa officinalis* L.) is a widely used medical plant belonging to the family Lamiaceae with sedative, carminative, antispasmodic, antibacterial and antiviral properties, mainly due to the content of essential oil (citral, citronellal) and phenolic compounds. In order to assess the impact of elevated background levels of O3 which are considered to be as harmful as episodic O3 peaks, *M.officinalis* plants were exposed to low ozone (O3) dosages (80 ppb) for 5 h. At the end of fumigation no visible symptoms were detectable, but necrotic lesions were observed later on. The biosynthesis of rosmarinic acid (RA) includes eight enzymes, e.g. 4-coumarate:coenzyme A ligase (4CL), phenylalanine ammonia-lyase (PAL), tyrosine aminotransferase (TAT) and rosmarinic acid synthase (RAS). The specifc activities of PAL and RAS as well as the RA content decreased significantly at 3 h FBE to ozone and were restored or even up-regulated at 5 h and 12 h FBE. The transcript levels of PAL, 4CL, TAT and RAS were investigated by quantitative RT-PCR in relation to the housekeeping gene EF1α. There was a rapid up-regulation of all genes at 3 h of ozone exposure, but at 24 h from beginning of exposure (FBE) only RAS and PAL were up-regulated. The aim of this study was to assess whether *M. officinalis* could be considered as an ozone-bioindicator species related to the influence of abiotic stress on secondary metabolism.

POSTER 184 – SESSION 8.4

Pyrrolizidine Alkaloids in Culinary Herbs

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Pyrrolizidine alkaloids (PAs) are toxic secondary plant compounds which are constitutively expressed as chemical defense strategy in some plant families. So far, more than 400 individual structures isolated from more than 560 plant species are known [1]. PAs are solely found in the angiosperms and the occurrence is mainly restricted to the Asteraceae (Senecioneae and Eupatorieae), the Boraginaceae, the Apocynaceae and the genus Crotalaria within the Fabaceae [1]. 1,2-unsaturated PAs are in general toxic. They are rapidly absorbed via the gastrointestinal tract and distributed in the body. Studies of PA metabolism in different model systems revealed the formation of reactive pyrrolic species inducing toxic and genotoxic, in particular, carcinogenic effects. In the light of the above, the European Food Safety Authority (EFSA) has published a scientific opinion on PAs in food and feed [1]. Here, we report the development and validation of a new HPLC-MS/MS sum parameter method to quantify low levels of 1,2-unsaturated PAs in complex food matrices. The new method was applied to analyze the PA-content of culinary herbs mixtures (supermarkets /farmer markets) and leafs/flowers of *Borago officinalis* and *Symphytum officinale* which are commonly used today in the revival of the so called "wild herbs cuisine/Wildkräuterküche".

[1]http://www.efsa.europa.eu/de/efsajournal/pub/2406.htm

POSTER 185 – SESSION 8.4

Identification of candidate genes involved in flavonolignan biosynthesis in *Silybum marianum*

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Flavonolignans are composed of a flavonoid and a phenylpropanoid part which are produced by a few angiosperm species. The flavonolignan mixture silymarin can be found in *Silybum marianum* (milkthistle, Asteraceae) and is known for its hepatoprotective properties and cytotoxic effects. Although the crucial enzymes in the biosynthetic pathway leading to dihydroflavonols and phenylpropanoids are already known, we lack information about the enzyme(s) responsible for the coupling and thus the formation of the silymarin components. It is assumed that this reaction might take place via a radical mechanism. Therefore, radical-forming enzymes like peroxidases (POD) or laccases may catalyse the coupling of taxifolin (2,3-dihydroquercetin) and coniferyl alcohol. In milk thistle this reaction is not strictly stereospecific because diastereomers (e.g. silybin A/B, isosilybin A/B) occur, however with enantiomeric excess. The relations between the different diastereomers differ in varieties and habitat. This might suggest an involvement of dirigent proteins (DIRs) regulating the enantiomeric ratio. Suspension cultures of *Silybum marianum* were established and enzyme assays with crude extracts and two commercial enzymes analysed by HPLC with respect to the formation of silymarin components. Partial sequences of a POD and different DIRs have been isolated.

POSTER 186 – Session 8.4

Establishing *Daucus carota* as a model to study polyacetylene biosynthesis

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Polyacetylenes are fatty-acid-derived aliphatic, unbranched C11-C17 hydrocarbons with several C-C-triple bonds widely distributed in the Apiaceae, Araliaceae and Asteraceae. The aim of our project is to get a better insight into polyacetylene biosynthesis using *Daucus carota* (Apiaceae) and its polyacetylenes falcarinol and falcarindiol as a model. Falcarinol and falcarindiol have been reported to be formed from linoleic acid with crepenynic acid and dehydrocrepenynic acid as key intermediates. In order to identify biosynthetically active tissues, we have analyzed the polyacetylene-content in roots and seedlings of different Apiaceae-plants including wild forms and cultivars of *D. carota*. The cultivars have its highest concentration of falcarinol in the cortical parenchyma, falcarindiol is widely spread in all tissues except the woody portion. In the wild form only low levels of falcarinol can be found, while falcarindiol is dominating. Falcarinol and falcarindiol are also produced in root cultures and callus cell suspension cultures of *D. carota* which we have established successfully. As both compounds have been reported as phytoalexins, we have conducted elicitation experiments with fungal pathogens and chemical elicitors using tissue cultures and soil grown plants. Once elicitation has been achieved, we are going to use the system to detect and clone biosynthetic enzymes.

POSTER 187 – SESSION 8.4

Analysis of Arabidopsis nitrile-specifier protein (NSP) function in vivo

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Specifier proteins amplify the chemical diversity of the glucosinolate-myrosinase defense system of the Brassicaceae. In Arabidopsis the specifier protein family includes NSP1-NSP5 and the putative ancestor At3g07720. NSP1 is responsible for simple nitrile formation upon glucosinolate breakdown in rosette leaves. Question: What are the roles of individual NSPs? Methods: We analyzed NSP1-NSP5 transcript levels in Col-0 plants and glucosinolate content and breakdown product formation in different organs and developmental stages of T-DNA insertion mutants. In order to obtain sufficient root material and to analyze a possible function of At3g07720 in Fe homoeostasis, we used a hydroponic culture on glass beads. Results: Only the nsp1 k.o. mutant has a strongly decreased nitrile:isothiocyanate ratio in homogenates of roots and seedlings. In seeds, only nsp2-1 and nsp2-2 show this effect. When any of the NSPs or At3g07720 is knocked out, glucosinolate breakdown in seeds is strongly increased as compared to Col-0 with the highest increase in nsp3-1, nsp3-2 and nsp5. This is, however, not accompanied by a decreased nitrile:isothiocyanate ratio. Conclusions: NSP1 is responsible for simple nitrile formation in roots and seedlings while NSP2 provides the major nitrile-forming activity in seeds. NSP3 and NSP5 may play a role in regulation of myrosinase activity in seeds.

POSTER 188 – SESSION 8.4

MdLOX1a - A determinant of aroma formation in ripening apple fruit?

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Flavour is a biochemically and genetically highly complex trait having a decisive impact on apple fruit quality. Due to limited information available on its molecular-genetic basis it has been more a random product of breeding than the result of targeted breeding strategies in the past. Biosynthesis of aroma compounds, such as alcohols and esters, is directly associated with the metabolism of fatty acids and lipids. Linoleic and α-linolenic acid are catabolized to precursors of fruit esters either by β-oxidation or the lipoxygenase (LOX) pathway. Recently, a map based QTL analysis for key aroma volatiles revealed a LOX candidate gene in close proximity to a QTL cluster for ester-type volatiles. As expression of the gene, designated as *MdLOX1a*, is induced by ethylene it might play a role in the ripening process. Indeed, we observed increased expression levels in the cultivars Golden Delicious and McIntosh during fruit ripening and storage. Also, stereo- and regio-chemistry of hydroperoxy products produced by MdLOX1a with linoleic acid confirmed earlier results for the major products of LOX activity found in apple fruits after storage. Here, we discuss the contribution of MdLOX1a to the formation of aroma-relevant ester-type volatiles.

POSTER 189 – SESSION 8.4

Multi enzyme systems involved in astin biosynthesis and their use in heterologous astin production

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Aster tataricus is a plant native to Siberia, Japan and Northern China. The roots of *Aster tataricus* are tradionally used in Chinese medicinal herb teas due to antibacterial and antiviral activities of compounds present in these roots. Amongst the secondary metabolites which have been isolated from these roots are astins and astin derivatives. Astins have been shown to have anti-tumour activity indicating an use in cancer therapy. However, only very low amounts of astins can be isolated from the plant. It is also difficult to synthesise them chemically without environmental impacts. Astins are dichlorinated, cyclic pentapeptides containing non-proteinogenic amino acids indicating a biosynthesis via non-ribosomal peptide synthetases (NRPS) and the involvement of one or two halogenases in the biosynthesis. The aim of the project is to detect the genes of the astin biosynthesis "gene cluster" and to use the genes to enhance astin production in organ cultures like hairy roots or callus.

POSTER 190 – Session 8.4

Characterization of terpene glycosyltransferases from grapes (*Vitis vinifera*)

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Monoterpenes are an important group of volatile components of grapes and wine, but the majority of terpenes are found as non-volatile glycosylated molecules. However, there are no glycosyltransferases (GT) known in grapes, which show specificity towards monoterpenes. Based on gene sequences of monoterpene-GT from *Arabidopsis thaliana*, we screened *in silico* for homologous sequences in the *Vitis vinifera* genome. About 70 putatively annotated *V. vinifera* genes were found. Alleles of several candidate genes of different cultivars were expressed in *E. coli* and analysed for their activity against six aroma-relevant monoterpenes. The recombinant VvGT7 protein was found to glycosylate the substrates nerol, citronellol and geraniol while terpineol, 8-hydroxylinalool and linalool were not converted. Interestingly, two of the ten identified VvGT7-alleles showed no activity at all. By sequence alignment we identified three amino acids which distinguish the active from the inactive proteins. Site-directed mutagenesis of these positions was performed to verify the importance of these amino acids according to the enzyme activity. Additionally, we identified three other proteins which glycosylate aroma-relevant terpenes. These enzymes were also heterologously expressed and functionally characterized.

POSTER 191 – SESSION 8.4

Lignan biosynthesis in different *Linum* species

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Linum species are known to produce aryltetralinlignans like podophyllotoxin (PTOX) and 6-methoxypodophyllotoxin (6-MPTOX). Especially podophyllotoxin is an important substance in the synthesis of different cancer medicines. Yet the biosynthetic pathway of aryltetralinlignans has not been identified completely. As the natural resources of PTOX, *Podophyllum hexandrum* and *Podophyllum peltatum* are getting rare, alternatives need to be found. These alternatives could include, e.g. biotechnological production of the compound. But this can only be done if all steps of the biosynthesis are known. Only the early steps of the aryltetralinlignan biosynthesis from coniferyl alcohol to matairesinol have already been described. Further steps involve several enzymes, which transfer a methyl group, e.g. the conversion of ß-peltatin to peltatin-A methyl ether. As methyltransferases play an important role, they need to identified and analyzed. The search for new methyltransferases is performed with suspension cultures of *Linum nodiflorum*, *Linum flavum* and *Linum album*, which accumulate aryltetralinlignans. These will be used to isolate cDNAs encoding methyltransferases, which will then be characterized on the molecular and biochemical level.

POSTER 192 – SESSION 8.4

METABOLIC ENGINEERING AND EVOLUTIONARY ANALYSIS OF PHYTOALEXIN RESPONSE IN BRASSICACEAE INCLUDING *THELLUNGIELLA*/EUTREMA AS A NEW MODEL SYSTEM

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Pathogens induce the biosynthesis of indolic phytoalexins in Brassicaceae. Approx. 50 distinct compounds are known, each of them typically restricted to a few species. They are synthesized according to two different principles. For example, camalexin of *Arabidopsis thaliana* is formed directly from the tryptophan-derivative indole-3-acetaldoxime (IAOx) via dehydration to the corresponding nitrile IAN. In addition, in Arabidopsis indole-3-carboxylic acid derivatives are synthesized via these intermediates and here we present evidence that Aldehyde Oxidase 1 is involved in this process. In contrast, the indolic phytoalexins of *Thellungiella salsuginea* (= *Eutrema salsugineum*) are synthesized more indirectly. Here, IAOx is first converted to indole glucosinolates, from which then 1-methoxybrassinin and wasalexins are produced. We have conducted a comparative transcriptomics approach to identifiy genes highly upregulated by stresses which induce phytoalexin biosynthesis. In addition we have introduced genes of camalexin biosynthesis with the aim to engineer phytoalexin profiles.

POSTER 193– SESSION 8.4

Formation of ellagic acid precursors in Fragaria

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Strawberry fruit (*Fragaria x ananassa*) contains high amounts of bioactive polyphenols, such as ellagic acid/ellagitannins. Ellagitannins not only possess strong antioxidant properties and antimicrobial activity, but also provide effective protection against human diseases including cardiovascular diseases, neurodegenerative diseases and hormonal cancers. Although the biological importance of these polyphenols is widely approved, the genetic background of their biosynthetic pathway is nearly unknown. Primary goal is the identification of genes and enzymes participating in the formation of 1,2,3,4,6-pentagalloylglucose, the proposed precursor of gallotannins and ellagitannins. The first specific metabolite in the biosynthesis of hydrolysable tannins is β-glucogallin (1-O-galloyl-β-D-glucopyranose), which is suggested to be generated by esterification of gallic acid and UDP-glucose as activated substrate. Therefore putative gallate:UDP-glucose glucosyltransferases possibly catalyzing the formation of β-glucogallin have been cloned and heterologously expressed in *E.coli*. The recombinant proteins were analyzed in radiochemical assays for their activity towards certain substrates including benzoic- and cinnamic acid derivatives, in particular gallic acid.

POSTER 194 – Session 8.4

Production of Bioactive Compounds in Non-Toxic Hairy Roots of Comfrey (Symphytum Officinale)

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Pyrrolizidine alkaloids (PA) have been found in many plant species, mainly in the families Asteraceae, Boraginaceae and Fabaceae. During metabolisation, these substances form derivatives which can harm human liver, capillaries and lung. Mutagenous and probably carcinogenous effects have also been shown. The use of medical plants like *Tussilago*, *Petasites* or *Symphytum* is thereby limited to application on the skin or requires complex cleaning processes. Medical plant extracts contain the main active compound or compounds and many additive substances that work as adjuvants. Often the extract is more effective than the isolated main compound, which means that synthesizing these substances does not solve the toxicity problem. The aim of the presented project is the downregulation of pyrrolizidine alkaloid biosynthesis in *Symphytum officinale* Hairy roots. RNA interference techniques will be used to gain less toxic cultures for secondary metabolite production, especially extracts containing high amounts of the anti-inflammatory compound allantoin.

POSTER 195 – SESSION 8.4

Secondary metabolism in sequenced algae: A survey of polyketide synthase genes

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It is well known that higher plants as well as algae produce a variety of secondary metabolites such as isoprenoids or phenolic compounds. Many secondary metabolites play important roles in the interaction of an organism with its biotic or abiotic environment. In algae, not much is known about the biosynthetic pathways and specific functions of secondary metabolism. Compared to macroalgae, fewer natural products have been isolated from microalgae even though the latter can serve as a source of sufficient and well-defined biological material from laboratory cultures. In addition, growing information from fully sequenced genomes and continuously refined genetic methods support the study of secondary metabolism in microalgae. An analysis of available genome sequences from ca. 30 algae suggests that the biosynthetic potential of microalgae is underestimated. For example, putative type I polyketide synthases (PKS), which are large multi-domain enzymes that produce polyketides, were found in approximately half of species. However, the existence of these enzymes and the resulting polyketides have not been verified experimentally, and their function remains enigmatic. Evolutionary implications of these findings and first experimental results will be discussed.

POSTER 196 – SESSION 8.4

Alpha-Tocopherol Production in Sunflower Callus Cells and Hairy Roots: A Genetic Approach

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Alpha-tocopherol is a secondary metabolite contained in almost every plastid containing tissue of plants. It has anti-oxidative properties and is considered as essential vitamin in humans. Thus, it is used as an additive in food, cosmetics and pharmaceutical products. The aim of the presented work is to produce large amounts of *all-R*-alpha-tocopherol by using genetically-engineered sunflower (*Helianthus sp.*) in vitro cultures in bioreactors. Only biological systems can produce this enantiomer exclusively. Subsequent downstream processing will yield the metabolite. The metabolism will be altered by overexpression of *Arabidopsis thaliana* L. gene VTE1 (tocopherol cyclase) and VTE4 (homogentisate phytyl transferase) in sunflower cultures, which already contain low amounts of the compound, under the control of the CaMV35S2x promoter. Ultimately, the increased enzyme levels should lead to an increased production of alpha-tocopherol, which will be determined by gas chromatography-mass spectrometry (GC-MS). In the future a highly productive sunflower hairy root culture will be established, cultivated and used for producing alpha-tocopherol.

Keywords: Sunflower, alpha-tocopherol, callus culture, Agrobacterium, VTE1, VTE4

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POSTER 197 – SESSION 8.4

Biochemical characterization of the *Arabidopsis thaliana* 3-phosphoglycerate dehydrogenase isoenzyme family.

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The 3-phosphoglycerate dehydrogenase (PGDH) catalyses the rate limiting reaction of the so called phosphorylated-serine pathway (PS-pathway). In many organisms like e.g. *E.coli* PGDH activity is tightly regulated by feedback inhibition through serine, the final product of the pathway. Although, genes encoding for all enzymes of the PS-pathway are present in plants, the function of this pathway still remains elusive. In *A. thalinana* PGDH activity is represented by three putative isoenzymes; however, a detailed biochemical characterization of theses enzymes is still missing. Therefore, the respective genes were cloned, heterologously expressed in *E. coli* and the purified proteins were subsequently characterized with respect to substrate affinity and regulation via serine feedback inhibition. Our results indicate that all three putative PGDH isoenzymes posses PGDH activity and the specific activity as well as the substrate affinity of the three isoenzymes do not differ significantly. In addition, only PGDH1 and PGDH3 were sensitive against feedback regulation at physiological serine concentrations, whereas PGDH2 remains completely insensitive. Taken together, our results shed new light on the regulation of the PGDH activity in plants.

POSTER 198 – Session 8.4

Molecular Investigation of Prenyltransferases Involved in Hyperforin Biosynthesis

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The diversity of plant secondary metabolites is due to the variety of biosynthetic pathways and downstream modifying enzymes, such as prenyltransferases (PTs), which add dimethylallyl and geranyl residues. In the medicinal plant *Hypericum perforatum*, PTs take part in the unexposed biosynthesis of hyperforin, the major active agent in extracts used for depression. Identification and characterization of PTs are necessary to understand the production of hyperforin and related compounds. A pool of recombinant PTs may provide biosynthetic modifications and creation of novel prenylated structures. cDNAs for putative PTs were isolated from *H. perforatum* using degenerate PCR primers derived from conserved domains. PCR products were completed using RACE-techniques, yielding four ORFs. Two additional full-length cDNAs were cloned from elicitor-treated *H. calycinum* cell cultures accumulating polyprenylated xanthones. All six cDNAs were expressed in *E. coli* and yeast, however, the recombinant enzymes did not act on the substrates used. After expression in insect cells (*Sf9*), one candidate was found to prenylate the substrate hyperxanthon E. Organic synthesis of new putative substrates is in progress as well as truncation of putative transit peptides for expression in yeast and insect cells. New PT genes identified recently in *H. perforatum* transcriptomes will be added to these analyses.

POSTER 199 – SESSION 8.4

Tropinone reductase-like enzymes from *Arabidopsis thaliana* - is it possible to change their coenzyme specificity?

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The genome of *Arabidopsis thaliana* encodes 16 genes, whose sequences show a high identity to known tropinone reductases (TRs) from Solanaceae. On this account the corresponding enzymes have been classified as tropinone reductase-like enzymes (TRLs). TRLs belong to the short-chain dehydrogenase/reductases (SDRs), offering the typical sequence size, the Rossmann fold in the N-terminal section and the catalytic triade in the C-terminal area. The TRLs from *A. thaliana* show a wide acceptance for different substrates like small flexible lipophilic carbonyls, cyclic monoterpenes and steroids. The reduction is often stereospecific and the corresponding alcohol oxidation stereoselective. These abilities make TRLs to be interesting candidates for industrial biocatalysis. We attempt to improve the catalytic qualities of TRLs using protein engineering.

The TRLs need NAD(P)(H) as coenzyme for their biocatalytic reactions. As NADP(H) is more expensive than NAD(H), one goal is to change the coenzyme specifity to NAD(H) by site directed mutagenesis. The mutagenesis experiments were planned by comparison of existing SDR-sequences, literature research and modeling studies.

POSTER 200 – SESSION 8.4

Overexpression of bacterial halogenases in *Arabidopsis thaliana*

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Over the last decades natural products became more important for many areas of medicine, pharmacology and agriculture. Many important antibiotics and anticancer agents are based on such secondary metabolites from different organisms. The introduction of a halogen (halogenation) into a structure can improve the bioactivity and bioavailability. It is already known that the halogenated plant growth hormone 4-chlorindole-3-acetic acid (e.g. synthesized in legumes) shows often a higher activity than indol-3-acetic acid. The introduction of a halogen in the metabolism of medically important plants could lead to a variety of novel plant metabolic products with improved properties. A regioselective incorporation of a halogen atom (chloride or bromide) can be achieved by using flavin-dependet halogenases. In this project three well characterised flavin-dependent halogenases will be introduced and overexpressed in *Arabidopsis thaliana* as a model plant. The project aim is to analyse changes in the metabolic profile of possible modified biosynthetic compounds in these plants with emphasis on chlorinated auxin derivatives. In addition, the effect of the presence of chlorinated indol-3-acetic acid on the growth of these plants will be analysed in detail.

POSTER 201 – SESSION 8.4

Drought Stress Boosts Secondary Metabolism

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Numerous studies document that plants exposed to drought stress reveal significantly higher concentrations of relevant natural products than identical plants of the same species cultivated under sufficient water supply. Unfortunately, up to now, the mechanisms behind this phenomenon remain unclear. Apparently, drought stress impacts on secondary metabolism in a general manner: Water shortage leads to stomata closure, restraining the influx of CO2. As consequence, far less reduced reduction equivalents (NADPH+H+) are consumed in the Calvin cycle. This restriction should cause an oversupply of NADPH+H+, boosting all metabolic processes that require reduction equivalents, e.g., the synthesis of secondary metabolites. When investigating this topic, special emphasis was put on the question, how drought stress impacts on plant metabolism. To elucidate the complex metabolic interactions, apart from the typical markers for drought stress, i.e., the expression of dehydrins, also the accumulation of secondary metabolites, as well as the Red/Ox state (% Glutathionox) had been determined for various spice and medicinal plants exposed to drought stress.

POSTER 202 – Session 8.4

The bHLH transcription factors MYC2, MYC3 and MYC4 interact with R2R3 MYBs to regulate the production of indolic and aliphatic glucosinolates in *Arabidopsis*

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MYB51 is a central regulator of indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. As R2R3 MYB transcription factors are usually known to act in concert with other regulatory proteins, the screening of cDNA library in yeast two-hybrid (Y2H) system was performed to find the putative interaction partners of MYB51. This Y2H screen, among others, revealed an interaction of MYC3 transcription factor with MYB51. MYC3 belongs to a small subclade of bHLH proteins consisting of MYC2, MYC3, MYC4 and MYC5. These BHLH factors are known to be important in jasmonate (JA) signalling. We suggest their involvement in JA-induced indolic glucosinolate accumulation via transcriptional regulation of the MYBs. Protein interaction studies (*in vitro* pull down and *in vivo* split YFP experiments) confirmed an interaction of all four BHLH-proteins with MYBs regulating the biosynthesis of indolic and aliphatic glucosinolates. In accordance with these findings the triple *myc2myc3myc4* mutant is devoid of all glucosinolates. Altogether our results revealed the novel function of MYCs in the regulation of indolic and aliphatic glucosinolates in *Arabidopsis thaliana*.

POSTER 203 – SESSION 8.4

AT3: The missing link in the evolution of PIP reductases?

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The family of the PIP reductases got its name from the first members found, namely the pinoresinol-lariciresinol reductases (PLRs), isoflavone reductases and phenylcoumaran benzylic ether reductases (PCBERs). Although a few members of this family are well characterised and some crystal structures are available some questions concerning the determination of the substrate specificity and its evolution remained open. Therefore we screened the genome of *Arabidopsis thaliana* for candidate genes encoding PIP reductase family members. We could show that AT3 is the first example of a PIP reductase combining PCBER and PLR activity within one protein. We will present the data on the biochemical characterisation of AT3 with respect to this double functionality and its enantiospecificity. In addition, the crystal structure of AT3 helps us to create mutations with the goal to figure out which amino acids/structural elements are responsible for the overall substrate specificity and the enantiospecificity of PIP reductases.

POSTER 204 – SESSION 8.4

Analysis of biotin biosynthesis and transport during vegetative growth, pollen tube elongation and embryo development

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Biotin (vitamin B7 or H) is an essential prosthetic group for enzymes catalyzing carboxylation, decarboxylation and transcarboxylation reactions. Biotin biosynthesis in plants is accomplished by four consecutive steps, which are catalyzed by four enzymes: BIO4, BIO1, BIO3 and BIO2. In this project we analysed the tissue and development-specific expression of the biotin biosynthesis genes *BIO1/3*, *BIO2* and *BIO4*. Furthermore we investigated a possible transport of the cofactor biotin into the pollen tube and into the embryo. To answer these questions we created *promotorBIO/GUS* and *promotorBIO/GFP* lines and analysed GUS staining and GFP fluorescence in these plants. Additionally we compared wild type (WT) and *bio1* mutant pollen tube growth under different biotin concentrations. These analyses revealed that biotin biosynthesis genes were expressed highly tissue specific. We found the highest expression in the vascular tissue of shoots and roots and in developing flowers. Consistently we observed differences in the growth between WT and *bio1* pollen tubes. These results indicate that these tissues maintain a high amount of the cofactor that can be made available to different biotin dependent enzymes and processes.

POSTER 205 – SESSION 8.4

Arabidopsis as a tool to study carotenoid homeostasis

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The carotenoid levels in plant tissues reflect steady-state concentrations determined by the rates of biosynthesis diminished by non-enzymatic carotenoid (photo)oxidation and enzyme-mediated catabolism catalyzed by carotenoid dioxygenases (CCDs). To investigate processes affecting carotenoid homeostasis we established a callus-based system developed from Arabidopsis seedlings. In dark-grown callus, carotenoid breakdown dominated over synthesis resulting in large net carotenoid losses. These did not depend on major CCDs but were rather due to non-enzymatic carotenoid degradation. Increased pathway fluxes by overexpression of the rate-limiting enzyme phytoene synthase (PSY) were compensated by enhanced carotenoid breakdown, but only at moderate fluxes. Higher rates of synthesis counteracted carotenoid breakdown and resulted in beta-carotene crystal formation. Hence, we hypothesize this sequestration mechanism to represent a major determinant of carotenoid stability reducing their accessibility towards catabolic processes. We applied the system to investigate the function of the cauliflower *OR* allele, known to foster crystal formation. Overexpressing lines showed significantly increased callus carotenoid contents and PSY protein levels compared to wild type. This suggests that OR determines carotenoid levels in part by modulating PSY protein turnover rates.

POSTER 206 – SESSION 8.5

Transorganellar coordination of β-oxidation between peroxisomes and mitochondria

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The importance of plant peroxisomes in seedling establishment is well known. At this stage peroxisomal β-oxidation enzymes operate to mobilize storage oil and activate pro-auxins to growth-promoting signaling molecules. In carbon starvation conditions such as extended darkness peroxisomal metabolism supplies the cell with carbon by fatty acid degradation. In senescence fatty acids are also broken down to redistribute membrane-lipid stored energy to sink tissues. Proteomics analyses of highly pure peroxisome isolations identified numerous matrix enzymes, but provided little information about membrane proteins. Especially with regard to metabolite transporter the peroxisomal membrane remains as one of the least characterized. To this end we studied the physiological role of putative peroxisomal membrane proteins in *Arabidopsis thaliana*. Expression analysis of a peroxisomal membrane protein revealed highest transcript abundance in senescing leaf tissue. Transgenic introduction of artificial micro RNA targeting this transcript resulted in plant lines that demonstrate accelerated leaf senescence and sensitivity to extended darkness. Mutant feeding experiments and metabolite profiling revealed the metabolic implications of this protein. We propose this protein to transport β-oxidation related molecules originated from plastids and mitochondria and unraveled the molecular nature of transport substrates and transport mode of this new player in central carbon metabolism.

POSTER 207 – SESSION 8.5

MAMP-induced activation of plasma membrane anion channels

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Guard cells are not only sensing abiotic signals, but also enhance the fitness of plants by responding to micro organisms. We examined early responses of guard cells to pathogenic fungi, by placing conidia of powdery mildew in close proximity of stomata. Life cell imaging showed that fungal growth and development inhibits stomatal opening. Using intracellular micro electrodes, we could link the inability of stomata to open, to an increased activity of guard cell anion channels. The high activity of anion channels impairs light-induced hyperpolarization of the guard cell plasma membrane. Because of the likely role of Microbe Associated Molecular Patterns (MAMPs), a major elicitor derived from fungal cell walls was applied by nano infusion through open stomata. Stimulation with chitosan activated guard cell anion channels and induced stomatal closure. We are currently testing the ability of guard cells to discriminate between MAMPs of different micro organisms. On the meeting new insights gained with these studies will be presented and implemented in a model that links MAMP-receptors to S-type anion channels.

Koers S, Guzel-Deger A, Marten I, Roelfsema MRG (2011) Barley mildew and its elicitor chitosan promote closed stomata by stimulating guard-cell S-type anion channels. Plant J. 68: 670-680

POSTER 208 – SESSION 8.5

Synthesis, transport and distribution of mono-, di- and polysaccharides in *Ajuga reptans* (*Lamiaceae*)

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Long distance transport of metabolites takes place in the sieve tube/companion cell complex (phloem). Metabolites can enter the phloem via apoplastic or symplastic loading. *A. reptans* is classified as exclusive symplastic phloem-loader because of its typical symplastic companion cells (intermediary cells) and sugar transport was not affected after PCMBS treating. But further studies showed that some symplastic loading plant species possess additional apoplastic phloem loading. The aim was to prove the loading mechanisms of *A. reptans*. Therefore the distribution of saccharides in different plant tissues and subcellular compartments under changing physiological parameters was investigated. The results show sucrose as an important storage carbohydrate, but stachyose is storage as well as transport carbohydrate. Variations in the sugar composition after changing light and temperature were observed. The enzymes involved in these processes, raffinose-/stachyose-synthase, will be characterized. Though *A. reptans* translocates raffinose and stachyose in the phloem, a full length cDNA of a sucrose transporter was identified. Its expression at different physiological treatments can now be analyzed by quantitative PCR. The role of sucrose transporter of putative symplastic phloem loaders and the general occurrence of mixed apoplastic-symplastic phloem loading will be discussed.

POSTER 209 – SESSION 8.5

Identification of Arabidopsis genes involved in ammonium sensing and signalling

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Ammonium (NH4+) is an important N source for plants. Interestingly, NH4+ affects also the root system architecture (RSA). NH4+ represses the elongation of the primary root and affects lateral root initiation and emergence. A local NH4+ supply leads to the formation of third order lateral roots. Previous studies lead to the assumption that the ammonium transporters (AMTs) are involved in the NH4+-mediated sensing and signalling cascade that leads to this changes in root phenotype. In order to investigate the molecular mechanism underlying NH4+-induced third order lateral root initiation and emergence and to identify additional members of the NH4+ mediated sensing and signalling cascade, auxin related *Arabidopsis* mutants will be analysed under local NH4+ supply.The auxin fluxes were altered and the auxin related transporters were analysed with regard to their effect on third order lateral root formation.

POSTER 210 – SESSION 8.5

The role of sodium in development of *Arabidopsis thaliana*

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Salt tolerance has been studied in detail in angiosperms for many years. In contrast, the effects of salt- and corresponding sodium-limiting conditions have not been addressed yet in depth. In order to investigate the biological significance of sodium in plants we tested the development and performance of the model plant *A. thaliana* under sodium-limiting conditions. Plants grown on MS medium almost depleted of sodium show strong growth retardation and chlorosis. Our results suggest that a critical amount of sodium is indeed essential to allow germination and successful seedling establishment. To unravel which metabolic and developmental processes are sodium-dependent we perform comparative transcriptome analyses. We expect that expression levels of genes involved in pathways affected by sodium starvation are altered under limiting conditions. Supported by metabolic analyses we will be able to identify metabolic enzymes. Further we will expect to identify and characterize new transport processes driven by sodium symport or antiport.

POSTER 211 – SESSION 8.5

Free fatty acids involved in lipid trafficking between ER and plastids

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Fatty acids are essential components of cellular life. They are key elements of membrane and storage lipids and certain fatty acids are precursors of signal substances. To become metabolically available free fatty acids require chemical activation by coenzyme A or ACP. In Arabidopsis a small gene family is encoding nine long chain acyl CoA synthetases (LACS) which are involved in establishing cellular acyl-CoA pools. Given the fundamental roles of these pools for lipid metabolism in general limitations of LACS activity was expected to impact various aspects of plant development. Surprisingly, mutant lines for individual LACS genes revealed no severe symptoms suggesting at least partially overlapping activities. To test this hypothesis and to reveal biological roles of individual LACS activities we combined mutant alleles systematically to establish a collection of double, triple, and quadruple mutant lines. Several mutant lines of this set allowed us to assign specific functions to individual LACS proteins. In this paper we describe a double mutant line compromised in fatty acid channeling between endoplasmic reticulum and the plastid. The mutant plants show severe morphological phenotypes which could be directly linked to biochemical deficits of the fatty acid metabolism. Our results support a modified model for lipid trafficking between endoplasmic reticulum and plastids in plant cells based on the re-synthesis of lipid precursor molecules.

POSTER 212 – SESSION 8.5

A Small Family of CCC1-like Proteins Mediate Vacuolar Iron Transport in Arabidopsis

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Iron deficiency is a nutritional problem in plants and reduces crop productivity, quality and yield. We have investigated the function of five Arabidopsis CCC1-like proteins with homology to the Vacuolar Iron Transporter-1 (AtVIT1). Heterologous expression of Vacuolar Iron Transporter-like-1 (*AtVTL1*; At1g21140) or *AtVTL2* (At1g76800) in the yeast Fe transport mutant, *Δccc1*, restored the ability to grow in the presence of 5 mM Fe. Isolated vacuoles from yeast expressing either gene in the *Δccc1* background had increased Fe content compared to vacuoles from the untransformed mutant. Transformation of the yeast *Δsmf1* (vacuolar Mn2+ transporter) or Δ*prm1* (Golgi P2a-type, Ca2+/Mn2+ ATPase) mutants with *AtVTL1* also led to complementation. In plants, transiently expressed GFP-tagged AtVTL1 was localized exclusively on the vacuolar membrane of onion epidermis cells, whereas GFP-tagged AtVTL2 was localized on the vacuole among other cellular membranes. When expressed under the 35S promoter in the *nramp3/nramp4* background, both *AtVTL2* and *AtVTL5* (At3g25190) complemented the Fe-dependent growth phenotype of the mutant. However, over-expression of *AtVTL1* was without effect. The Fe concentration in seeds in the *nramp3/nramp4* mutant overexpressing *AtVTL1*, *AtVTL2* and *AtVTL5* was between 50 and 60% higher than in the non-transformed double mutant or the wild-type control. We concluded that the VTL proteins are vacuolar divalent cation transporters and that AtVTL1, AtVTL2 and likely AtVTL5 transport Fe into the vacuole.

POSTER 213 – SESSION 8.5

Characterization of fatty acid transport in *Synechocystis* sp. PCC 6803

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The transfer of fatty acids is an essential, however largely uncharacterized process at the membranes of several higher plant organelles like peroxisomes, the endoplasmatic reticulum, or chloroplasts. Here, we apply the cyanobacterium *Synechocystis* sp. PCC 6803 as a model system to identify the molecular components involved in fatty acid transport. Loss-of-function mutants of *Synechocystis* are analyzed regarding the response to exogenous α-linolenic acid. An altered response to this fatty acid likely is caused by changes in fatty acid transport activity of the cell. Therefore these mutants are then used to investigate the molecular components and mechanisms involved in fatty acid transport over the membrane. In order to extend these studies to the situation in the plastids of higher plants, these *Synechocystis* mutants are then to be complemented with candidate genes from *Arabidopsis*. A successful complementation will yield good candidates for the molecular components of the fatty acid transport machinery in the plastid.

POSTER 214 – SESSION 8.5

A transport protein involved in cofactor supply for plant peroxisomes

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Plant peroxisomes are highly dynamic organelles involved in a wide range of metabolic processes that are critical for plant growth and development, including fatty acid β-oxidation and the glyoxylate cycle, photorespiration, biosynthesis of biotin and phytohormones, and detoxification of reactive oxygen species (ROS). Most of these enzymatic reactions require cofactors, such as ATP, NAD, and CoA. Since the cofactor biosynthesis is located outside peroxisomes, transporter proteins are needed to supply these metabolic pathways with ATP, NAD, and CoA. We have identified a peroxisomal carrier, PXN (Peroxisomal NAD carrier), which catalyzes the import of NAD. Interestingly, in vitro uptake experiments revealed that PXN accepts - besides NAD - NADH and CoA as substrates. This broad substrate specificity is unique compared to other known NAD carriers in plastids and mitochondria. On structural level PXN exhibits an enlarged loop region between the third and fourth transmembrane helix, which is absent in other NAD transporters. Ongoing experiments will show the influence of this domain and the contained phosphorylation site on substrate specificity and transport activity

POSTER 215 – SESSION 8.5

The *Dionaea muscipula* ammonium channel *DmAMT1* provides NH4+ uptake associated with Venus flytrap's prey digestion

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Background: Ammonium transporter (AMT/MEP/Rh) superfamily members mediate ammonium uptake and retrieval. This pivotal transport system is conserved among all living organisms. For plants nitrogen represents a macronutrient available in the soil as ammonium, nitrate, and organic nitrogen compounds. Plants living on extremely nutrient-poor soils have developed a number of adaptation mechanisms including carnivorous life style. This study addresses the molecular nature, function, and regulation of prey-derived ammonium uptake in the Venus flytrap Dionaea muscipula, one of the fastest active carnivores. Results: The Dionaea muscipula ammonium transporter DmAMT1 was localized in gland complexes where its expression was upregulated upon secretion. These clusters of cells decorating the inner trap surface are engaged in i) secretion of an acidic digestive enzyme cocktail as well as ii) uptake of prey-derived nutrients. Voltage-clamp of Xenopus oocytes expressing DmAMT1 and membrane potential recordings with DmAMT1 expressing Dionaea glands exhibited the hallmark biophysical properties of a NH4+-selective channel. Conclusions: We suggest that regulation of glandular DmAMT1 and membrane potential readjustments of the endocrine cells provide for effective adaptation to varying, prey-derived ammonium sources.

POSTER 216 – Session 8.5

The molecular origin of the *Dionaea* action potential

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Since the time of Darwin, scientists are eagerly interested in the mechanisms of trap closure of the Venus flytrap, *Dionaea muscipula*. Prey-derived mechanical stimulation of trigger hairs elicit action potentials (APs) leading to trap closure. However, the molecular grounds for trap excitability remained obscure until today. Based on transcriptome analysis we screened for ion channels involved in the generation of APs. Among the identified ion channels, we cloned and analyzed the shaker K+ channel KDM (K+-transporter Dionaea muscipula). qRT-PCR experiments revealed a trigger hair-specific expression pattern for KDM. Electrical measurements in Xenopus oocytes showed that KDM represents a hyperpolarization-activated, inwardly rectifying K+ channel. Interestingly, KDM was characterized by a peculiar pH-sensitivity leading to enhanced activation of KDM at acidic condition. Our results suggest the following model: i) mechanical stimulation release cell wall bound protons into the trigger hair apoplast, ii) the sudden acidic burst activates KDM ion channels, iii) KDM-mediated K+ influx generates a receptor potential (RP), iv) if RP exceeds a certain threshold, anion channels are activated and further depolarise the PM, v) the AP propagates across the trap's lobes leading to trap closure. Thus, our results provide first insights into the excitability of the Venus flytrap.

POSTER 217 – SESSION 8.5

Arabidopsis Root Anion Channel SLAH2 Gains Nitrate Selectivity from Distinct Pore Phenylalanines

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Plants operate anion channels for anion translocation from the root to the shoot, for turgor-driven processes, and as key regulators of stress responses. Interestingly, the SLAC/SLAH anion channel family comprises individual members that exhibit distinct permeabilities for nitrate and chloride. Here we characterized the root-expressed SLAH2 anion channel that transports nitrate but excludes chloride. Due to its peculiar nitrate specificity and the pronounced difference to the chloride and nitrate permeable founder of the SLAC/SLAH family, SLAC1, we aimed on the molecular grounds of the distinct selectivity between SLAC1 and SLAH2. Based on structural models we identified two pore occluding phenylalanines present in both channels. Mutations within the pore region of SLAC1 and SLAH2 finally converted SLAC1 into a SLAH2-like nitrate-specific anion channel and vice versa. Thereby the pore occluding aromatic side chains were observed to be restricted in their flexibility by distinct residues located on TM3. These findings explain the distinct permeability of SLAC/SLAH anion channels and thus the peculiar nitrate selectivity of SLAH2.

POSTER 218 – SESSION 8.5

Malate import in plastid membranes

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Eukaryotic cells are compartmented. Therefore, complete understanding and manipulation of metabolism requires understanding of the transport proteins, which connect the compartments. Two bioenergetically interesting processes, C4 photosynthesis and fatty acid biosynthesis, require malate import into plastids.

In the leucoplasts of the oil storing seeds of *Ricinus communis*, fatty acids are synthesized from malate, which is imported from the cytosol. In the C4 cycle of NADP-malic enzyme plants, malate is imported into bundle sheath chloroplasts for at the site of RubisCO. The molecular identity of the transporter catalyzing that flux is currently unknown. Two complementary approaches are pursued to identify and characterize the malate transporter: a proteomic study of *Ricinus* leucoplast envelopes and a candidate protein approach based on transcriptomics data available for *Ricinus* and C4 plants.

A PHT family member was identified in transcriptomics data of *Ricinus* seed filling and verified by RNAseq in different NADP-ME plants. The protein was heterologously expressed in yeast and the biochemical characterization via uptake experiments in artificial proteoliposomes is under way. Once the molecular identity of the malate transporter is established, its potential rate-limiting role can be explored in C4 photosynthesis in C4 plants as well as in oil production in *Ricinus*.

POSTER 219 – SESSION 9.1

Functional characterization of Zeaxanthin-Epoxidase genes from *P. tricornutum*

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The pathway of carotenoid biosynthesis of *P. tricornutum* is only partly elucidated. The function of some important genes is still unknown, e.g. the ones encoding β-ionone ring hydroxylases and zeaxanthin epoxidases. For the latter gene, three putative reading frames can be found in *P. tricornutum*. Zeaxanthin epoxidase (Zep) is the enzyme that converts zeaxanthin via antheraxanthin into violaxanthin. This reaction is not only an important step towards the end product fucoxanthin, but also part of the VAZcycle and therefore responsible for NPQ. For the three annotated gene copies the function has not been demonstarted yet.

In the presented work, we set up an approach to show which copies are functional by genetic complementation of the *Arabidopsis thaliana* NPQ2 mutant in which Zep is strongly down-regulated. Functionality of Zeps from *P. tricornutum* could be demonstrated for copy #2 and #3 by their phenotype and by HPLC identification of the Zep products antheraxanthin and violaxanthin. This pathway results was supported by NPQ measurements as well as quantitative real time PCR analysis. In contrast to #2 and #3, copy #1 was unable to reconstitute epoxidation indicating that it is not functional. We assume that gene copy #1 is a pseudo gene.

POSTER 220 – SESSION 9.1

Cell-wall glycoproteins of the green alga *Scendesmus obliquus* related to the cell-wall polypeptide GP3B of *Chlamydomonas reinhardtii*

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The cell wall of the green alga *Scendesmus obliquus* contains chaotrope soluble glycoproteins and polypeptides cross-linked to the insoluble wall fraction. Three prominent polypeptides apparent molecular masses of 144, 135 and 65 kDa are released from the insoluble wall fraction by treatment with hydrogen fluoride (HF) in anhydrous pyridine. The 144- and 135-kDa components cross-react with a polyclonal antibody raised against the 100-kDa deglycosylation product of the chaotrope soluble cell-wall glycoprotein GP3B of *Chlamydomonas reinhardtii* [1]. A 135-kDa polypeptide immunochemically related to GP3B of *C. reinhardtii* is also a prominent deglycosylation product of the chaotrope-soluble cell-wall fraction of *S. obliquus*. Protein-blot analyses of the untreated chaotrope-soluble wall fraction of *S. obliquus* with this particular antibody reveals cross-reacting bands with apparent molecular masses of 135, 165, 170 and 240 kDa, the 170-kDa component being most prominent in the Coomassie-stained gels. The latter findings indicate that the 165-, 170-kDa and 240-kDa components are differentially glycosylated isoforms of the 135-Da polypeptide related to GP3B of *C. reinhardtii*.

[1] Voigt, J., Kieß, M., Getzlaff, R., Wöstemeyer, J. and Frank, R. (2010) Mol. Microbiol. 77: 1512-1526.

POSTER 221 – Session 9.1

A chloroplast ribonucleoprotein complex involved in group II intron trans-splicing

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Maturation of chloroplast *psaA* pre-mRNA from the green alga *Chlamydomonas reinhardtii* requires the *trans*-splicing of two split group II introns. Several nuclear-encoded *trans*-splicing factors are required for the correct processing of *psaA* mRNA [1]. Among these is the recently identified Raa4 protein, which is involved in splicing of the tripartite intron 1 of the *psaA* precursor mRNA [2]. Part of this tripartite group II intron is the chloroplast encoded *tscA* RNA, which is specifically bound by Raa4. Using Raa4 as bait in a combined tandem affinity purification and mass spectrometry approach, we identified core components of a multisubunit ribonucleoprotein complex, including three previously identified *trans*-splicing factors. We further detected *tscA* RNA in the purified protein complex, which seems to be specific for splicing of the tripartite group II intron. A yeast-two hybrid screen and co-immunoprecipitation identified chloroplast- localized Raa4-binding protein 1 (Rab1), which specifically binds *tscA* RNA from the tripartite *psaA* group II intron. The yeast-two hybrid system provides evidence in support of direct interactions between Rab1 and four *trans*-splicing factors.

[1] Jacobs J, Glanz S, Bunse-Graßmann A, Kruse O, Kück U (2010) Europ J Cell Biol 89: 932-939

[2] Glanz S, Jacobs J, Kock V, Mishra A, Kück U (2012) Plant J 69:421-431

POSTER 222 – SESSION 9.1

A VDL protein of the violaxanthin de-epoxidase superfamily catalyzes the formation of neoxanthin, an intermediate in the biosynthesis of the algal light-harvesting carotenoids fucoxanthin and peridinin

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Although diatoms and dinophytes are important contributors to marine primary production, the biosynthesis of their major light-harvesting pigments fucoxanthin and peridinin is largely unexplored. Based on experimental data and considering the molecular structure of both pigments, violaxanthin and neoxanthin are likely intermediates in their biosynthesis. Here, we have cloned two VDL (violaxanthin de-epoxidase-like) genes from the diatom *Phaeodactylum tricornutum* and the dinophyte *Amphidinium carterae* encoding proteins with similarity to the enzyme violaxanthin de-epoxidase (VDE) involved in the photoprotective xanthophyll cycle in land plants and many algae. In an *in vitro*-assay commonly used for VDE, we found VDL to isomerize violaxanthin to neoxanthin with a final ratio of 75% neoxanthin and 25% violaxanthin. Contrary to VDE, VDL required neither ascorbate nor any other cofactor. An *in silico*-analysis of the deduced protein sequences indicated the presence of tripartite targeting signals at their N-terminus suggesting that they localize to the thylakoid lumen. In line with this prediction, a biochemical characterization of the diatom VDL revealed that the enzyme is most active under acidic conditions between pH 5 and 6. Our results indicate that VDL is a novel enzyme catalyzing a central step in the formation of fucoxanthin in diatoms and peridinin in dinophytes.

POSTER 223 – Session 9.1

Genetic transformation of the diatom *Cyclotella meneghiniana*: *Fcp6* knock-down results in a decreased level of NPQ.

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Being one of the major components of marine phytoplankton, diatoms must cope with light fluctuations in a daily manner. NPQ is one of the mechanisms to dissipate excess light energy as heat thus protecting the photosynthetic apparatus from damage. Studies on *fcp6* showed its influence on fluorescence yield and it was thus hypothesised to play a role in NPQ (1). In order to study the influence of Fcp6 in vivo, wild type cells of the centric diatom *Cyclotella meneghiniana* were genetically modified by ballistic transformation. This was carried out using two vectors (co-transformation), the first vector carrying an antisense construct complementary to the 5'-UTR region of the *fcp6* gene and the second one providing *nat* resistance as a selective marker, like previously used for the transformation of *Thalassiosira pseudonana* (2). Our results on the *fcp6* knock-down mutants showed a severely impaired ability for NPQ, as well as a very slow growth rate compared to wild type cells, thus demonstrating the pivotal role of Fcp6 in NPQ.

(1) Gundermann, K., Büchel, C., 2012, Factors determining the fluorescence yield of fucoxanthin-chlorophyll complexes (FCP) involved in non-photochemical quenching in diatoms, *BBA-Bioenergetics* 1817 (7), 1044-1052.

(2) Poulsen, N., Chesley, P.M. and Kröger, N., 2006, Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (Bacillariophyceae), *J. Phycol.* 42, 1059-1065.

POSTER 224 – Session 9.1

Characterization of a lycopene cyclase-fusion protein from the green alga *Ostreococcus lucimarinus* catalyzing the simultaneous formation of α-carotene and β-carotene

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Biosynthesis of asymmetric carotenoids such as α-carotene and lutein in plants and green algae involves two functionally different lycopene cyclases. The two cyclases are closely related and probably resulted from an ancient gene duplication. While in most plants and algae investigated so far the two cyclases are encoded by separate genes, prasinophyte algae of the order Mamiellales contain a single gene encoding a fusion protein with two cyclase domains and a C-terminal light-harvesting protein. We have cloned the gene and found that it catalyzes the simultaneous formation of α-carotene and β-carotene when expressed in *Escherichia coli*. Partial deletions of the linker region between the two cyclase domains resulted in an altered stoichiometry of the two products, suggesting that the overall structure of the fusion protein tunes the balance between the two enzymatic activities. The ratio between α-carotene and β-carotene could also be altered by gradual truncation of the C-terminus, suggesting that the LHC domain is involved in regulating the product stoichiometry in the algae, which would represent a novel regulatory mechanism. Moreover, the lycopene cyclase fusion protein may be useful for the biotechnological production of the asymmetric carotenoids α-carotene or lutein in bacteria or fungi.

POSTER 225 – Session 9.1

A Novel Cryptochrome in the Diatom *Phaeodactylum tricornutum* Influences the Regulation of Light Harvesting Protein Levels

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Diatoms possess several genes for proteins of the cryptochrome / photolyase family. A typical plant cryptochrome sequence was not found, but one protein groups with higher plant and green algal cryptochromes. This protein, CryP, binds flavin adenine dinucleotide (FAD) and 5,10-methenyltetrahydrofolate (MTHF) as demonstrated by spectroscopic studies on heterologously expressed protein. Binding of MTHF has previously been demonstrated for CPD photolyases and DASH cryptochromes. In recombinant CryP, the FAD chromophore is present in its neutral radical state and has a red-shifted absorption maximum at 637 nm, which is more characteristic for a DASH cryptochrome than a CPD photolyase. Upon illumination with blue light, the fully reduced state of FAD is formed in the presence of reductant. Knock-down *Phaeodactylum tricornutum* mutants of CryP showed increased levels of proteins of light harvesting complexes (Lhc), the Lhcf proteins, *in vivo*. In contrast, the levels of proteins active in light protection, Lhcx, were reduced. Thus, CryP cannot be directly grouped to known members of the cryptochrome / photolyase family. It is most similar in sequence to a plant cryptochrome and is involved in the regulation of light harvesting protein expression, but displays spectroscopic features and a chromophore composition most typical of a DASH cryptochrome.

POSTER 226 – SESSION 9.1

Insights into peroxisomes of diatoms

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Peroxisomes are single membrane bound and functionally related compartments. They are thought to be present in almost all eukaryotic cells, although the bulk of our knowledge about peroxisomes has been generated from only a handful of model organisms. Peroxisomal matrix proteins are synthesized cytosolically and thus are imported posttranslationally into the peroxisomal matrix. The import is generally thought to be mediated by two different targeting signals. These are recognized by the two import receptor proteins Pex5 and Pex7 respectively, which facilitate transport across the peroxisomal membrane. Here we show the first *in vivo* localization studies of peroxisomal proteins in a representative organism of the ecologically relevant group of diatoms. We demonstrate that peroxisomes in *Phaeodactylum tricornutum* differ regarding targeting signals of matrix proteins and significance for photorespiration compared to other model organisms.

POSTER 227 – SESSION 9.2

Genetic variability within the green-algal genus *Klebsormidium* (Streptophyta) in three Biodiversity Exploratories (Project SoilCrust)

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The genus *Klebsormidium* belongs to a group of green algae (Streptophyta), which are together with others closely related to the land plants. They are able to form characteristic biofilms and distributed in almost all terrestrial habitats from tundra of the Polar areas, to desert and arid regions of both sites of the equator. *Klebsormidium* is a typical representative of biological crusts, which play an important ecological role in primary production, nitrogen fixation, nutrient cycling, water retention and stabilization of soils. We collected from three large-scale and long-term research sites (Biodiversity Exploratories) in Germany (Schorfheide-Chorin, Hainich-Dün, and Schwäbische Alb) soil samples and isolated more than 170 strains of *Klebsormidium*. To detect the genetic diversity of these strains, we sequenced the internal transcribed spacer region of the nuclear ribosomal cistron (ITS-1, 5.8S rDNA, and ITS-2). The molecular phylogeny of the ITS rDNA sequences showed that the strains of *Klebsormidium* belong to only to two clades, which are designated as clades B/C and E sensu Rindi et al. (2011: Mol. Phylogenet. Evol. 58: 218-231). To discriminate *Klebsormidium* at species level, we used the secondary structures of ITS-2 rDNA sequences as DNA barcode marker and compared these results with the morphology of the described species. Interestingly, all isolates from grassland plots belong to the clades B/C (Barcode V), whereas all strains from different forests are members of the clade E (Barcodes VIII, IX, and XIV).

POSTER 228 – SESSION 9.3

How Chlamydomonas Suffers and Recovers from Heat Stress - a Systems Biology Approach

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To understand how *Chlamydomonas reinhardtii* acclimates to and recovers from thermal stress, we exposed cells to 42°C for 24 h and allowed them to recover at 25°C for 8 h. During this time we monitored protein dynamics via 15N shotgun LC-MS/MS proteomics allowing for the quantification of ~4000 proteins. Furthermore, we analysed levels of ~70 polar (GC-MS) and ~200 lipophilic metabolites (LC-MS). We also monitored cytological parameters and major cellular components like cell size, cell number, cellular ultrastructure (EM), DNA- and protein content. We observed a rapid cell cycle arrest and reduced growth at the onset of heat stress, which came along with a reduced accumulation of ribosomal proteins and a depletion of metabolite pools of the central metabolism. We observed the induction of cellular protection and acclimation mechanisms like an increased accumulation of chaperones, ROS scavengers, compatible solutes and a rapid rearrangement of membrane lipid composition. We observed the accumulation of aberrant structures at regions where multiple thylakoid membranes emerge, presumably arising from disturbed photosystem biogenesis and thus explaining the discrepancy between increased levels of photosystem subunits and decreased oxygen evolution. Surprisingly, not all cellular adaptations to heat stress were reverted when cells started to divide again during recovery.

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